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Pursuant to 37 CFR 1.53(b), transmitted herewith for filing is the CIP patent application of application Serial No. 09/053,583 filed April 1, 1998 and Serial No. 09/408,646 filed September 30, 1999

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## Title: "ANTI-FAS ANTIBODIES"

Priority Claim (35 U.S.C. 119) is made, based upon:

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Hei 10-276882	September 30, 1998

## Enclosed herewith are:

- Specification (Description, Claims, Abstract): Pages 1 - 372; Number of claims 1 - 119
- Declaration and Power of Attorney (executed)
- 69 Sheets of drawings, Figures 1 - 73  Formal  Informal
- Assignment and Form PTO-1595 Recordation Form Cover Sheet and Check for \$40.00
- Certified copies of Hei 10-276881 and Hei 10-276882 (Certified copies of the first three priority documents were filed in SN 09/053,583 on July 22, 1998)
- COMPUTER READABLE FORM OF SEQUENCE LISTING, PAPER COPY OF SEQUENCE LISTING AND STATEMENT UNDER 37 CFR 1.821(f)
- INFORMATION DISCLOSURE STATEMENT, including Form PTO-1449
- SUBMISSION OF COPIES OF CERTIFICATES OF MICROORGANISM DEPOSITS
- Receipt Postcard

	<b>Number Filed</b>	<b>Number Extra</b>	<b>Rate</b>	<b>Calculations</b>
<b>Total Claims</b>	<u>212</u>	<u>-20</u> = <u>192</u>	x \$18.00	= <u>\$ 3,456.00</u>
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ANTI-FAS ANTIBODIES

This application is a continuation-in-part application of application Serial No. 09/053,583, filed April 1, 1998 and application Serial No. 09/408,646, filed September 30, 1999; the entire contents of said applications are hereby incorporated by reference herein.

Field of the Invention

The present invention relates to antibodies and fragments thereof, especially humanized antibodies, recognizing the Fas antigen, to DNA encoding all or part of such an antibody, and to agents, comprising such antibodies, for the prophylaxis and/or treatment of conditions arising from abnormalities in the Fas/Fas ligand system.

Background of the Invention

The physiological death of cells in a living organism in the natural course of events is known as apoptosis, and is distinguished from the pathological death of cells, i.e. necrosis [c.f. Kerr et al., (1972), Br. J. Cancer, 26, 239 et seq.]. Apoptosis is an example of programmed cell death, which is where certain cells are programmed, in advance, to die in a living organism in the natural course of events, such as when the cell in question has performed a pre-determined function. Apoptosis is characterised by such morphological changes as curved cell surface, condensed nuclear chromatin and fragmented chromosomal DNA, amongst others.

Apoptosis plays a role in the differentiation of lymphocytes (T cells and B cells) by eliminating cells that recognize an autoantigen. In this respect, it has been demonstrated that 95%, or even more, cells, such as those which react with autoantigens, are eliminated in the thymus during the maturation of T lymphocytes [c.f. Shigekazu Nagata, Tanpakushitsu Kakusan Koso, (1993), 38, 2208-2218]. When such cells are not eliminated by apoptosis, then it is believed that this is a cause of autoimmune disease, due to the presence of mature, auto-reactive lymphocyte

in the system [c.f. Nakayama et al., (1995), Mebio, 12 (10), 79-86].

Various molecules have been identified as being involved in apoptosis, including: Fas [c.f. Yonehara, S., et al., (1989), J. Exp. Med., 169, 1747-1756]; tumor necrosis factor receptor [c.f. Loetscher, H., et al., (1990), Cell, 61, 351-359]; CD40 [c.f. Tsubata, T., et al., (1993), Nature, 364, 645-648]; and perforin/granzyme A [c.f. Jenne, D. E., et al., (1988), Immunol. Rev. 103, 53-71].

Fas is a transmembrane protein, present on the cellular surface, and binding of its extracellular domain to a protein generally known as "Fas ligand", expressed on the surface of other cells, induces apoptosis in the cell expressing Fas. Abnormalities in the Fas/Fas ligand system result in various disorders, by failing to delete cells which could be detrimental to homeostasis, and which should have been eliminated by apoptosis, or, alternatively, by inducing apoptosis in cells not otherwise scheduled for elimination and which could be essential for maintaining homeostasis. Such disorders are those referred to herein as being conditions arising from abnormalities in the Fas/Fas ligand system.

In the development, or progression, of diseases arising from abnormalities of the Fas/Fas ligand, it is often the case that abnormal cells, which express Fas but which, nevertheless, remain undeleted (abnormal cells), either attack normal tissues or cells, or else proliferate abnormally, thereby causing disorders in the tissues or cells which, in turn, lead to the respective disease symptoms. In some cases, these disorders may arise from, or be exacerbated by, the expression of Fas on the abnormal cells, thereby stimulating apoptosis in normal tissues or cells. Specific examples of diseases attributable to abnormalities of

the Fas/Fas ligand system are as follows.

Autoimmune diseases.

Links between various human autoimmune diseases (Hashimoto disease, systemic lupus erythematosus, Sjögren syndrome, pernicious anemia, Addison disease, insulin dependent diabetes mellitus, scleroderma, Goodpasture's syndrome, Crohn's disease, autoimmune hemolytic anemia, sterility, myasthenia gravis, multiple sclerosis, Basedow's disease, thrombopenia purpura, rheumatoid arthritis) and abnormalities in the Fas/Fas ligand system have been reported many times.

In the mouse, various genetic abnormalities of the Fas/Fas ligand system are known, including the lpr (lymphoproliferation), gld (generalized lymphoproliferative disease), and lpr<sup>cg</sup> (where the lpr gene complements the gld gene) mutations. Mice having such genetic abnormalities all exhibit various autoimmune symptoms, accompanied by characteristic systemic swelling of the lymph nodes.

The MRL-lpr/lpr mouse, a mouse model of spontaneous human systemic lupus erythematosus, shows a marked increase in the mass of its lymph nodes and produces autoantibodies causing nephritis owing to the formation of immune complexes. It is speculated that this mouse exhibits this pathology as a result of a mutation in the Fas gene, resulting in a lack of immunological tolerance to autoantigen by failure of Fas induced apoptosis in the peripheral system, as well as by the long-term accumulation of activated autoreactive T cells [c.f. Strasser, A., Nature, 373, 385 (1995)].

In the human, several cases have been reported, including two pediatric cases involving swelling of the lymph nodes, hyper γ-globulinemia and marked increase in CD4<sup>+</sup>CD8<sup>+</sup> T cells [c.f.

Sneller, M.C., et al., (1992), J. Clin. Invest., 90, 334]. These cases were reported to be based on abnormalities in the Fas gene [c.f. Fisher, G. H., et al., (1995), Cell, 81, 935; and Rieux-Laucat, F., et al., (1995), Science, 268, 1347], and designated autoimmune lymphoproliferative syndrome (ALPS). Based on these findings, it is considered that the apoptosis-inducing system, mediated by Fas, is involved to a large extent in the establishment and maintenance of self-tolerance, not only in the mouse but also in the human, and disorders of this system induce various autoimmune diseases.

It is also known that rheumatoid arthritis has an autoimmune element, based on the fact that the vast majority of T cells invading affected regions of rheumatoid arthritis patients and causing tissue destruction express Fas [c.f. Hoa, T. T. M., et al., (1996), J. Rheumatol., 23, 1332-1337].

Many cases of insulin dependent diabetes mellitus result from a critical shortage of insulin secretion, owing to destruction of pancreatic beta cells by autoreactive T cells. Thus, elimination of autoreactive T cells is important in the radical treatment of certain forms of insulin dependent diabetes mellitus.

In graft versus host disease, such as occurs after a bone marrow transplant, expression of Fas increases in the affected organ, and there is a direct correlation between the degree of increase in Fas expression and damage to the target organ [c.f. Chu, J. L., et al., (1995), J. Exp. Med., 181, 393]. Therefore, the aim in preventing or treating this disease, is to block apoptosis in the cells of the target organ and to decrease the numbers of cells attacking the target organ.

### Allergic diseases

Inflammatory cells involved in allergic diseases are normally activated and invade the lesions. The inflammatory cells accumulate locally in the lesion, and are able to continue to function long term, as their lives are extended by suppression of apoptosis. In an experimental model in which acidophilic inflammation of the air passage is induced in mice, it has been demonstrated that administration of an anti-Fas antibody, having apoptosis-inducing activity, via the air passage, results in the disappearance of invasion of acidophiles under the mucosa normally seen after inhalation of the allergen [c.f. Tsuyuki, S., et al., (1995); J. Clin. Invest., 96, 2924]. Therefore, it is possible to alleviate the symptoms in allergic inflammation by inducing apoptosis in the inflammatory cells.

### Rheumatoid arthritis

Apart from the autoimmune aspect of rheumatoid arthritis described above, abnormally proliferating synovial cells in the lesions are known to express Fas [c.f. Hoa, T. T. M., et al., (1996), J. Rheumatol., 23, 1332]. Apoptosis can be induced by stimulating the synovial cells from such lesions with anti-Fas antibody having apoptosis-inducing activity [c.f. Nakajima, T., et al., (1995), Arthr. Rheum., 38, 485]. In other words, the Fas/Fas ligand system is not functioning properly in the foci of rheumatoid arthritis patients, and neither autoreactive T cells nor abnormally proliferating synovial cells are eliminated, despite both expressing Fas.

### Arteriosclerosis

Although the final diagnosis of cell deaths at the center of arteriosclerosis lesions is necrosis, involvement of apoptosis in the progression and degeneration processes has been reported [c.f. Kenji Harada (1997), Gendai Iryou, 29, 109]. Electron microscopy of the lesions of arteriosclerosis shows apoptosis of

smooth muscle cells, characterized by nucleic condensation [c.f. Isner, J. M., et al., (1995), Circulation, 91, 2703]. Further, it has been reported that foam cells, which are macrophages gathering in the inner layer of the artery and incorporating lipids in early arteriosclerotic lesions, express Fas and are caused to undergo apoptosis with naturally occurring apoptosis-inducing anti-Fas antibodies [c.f. Richardson, B. C., et al., (1994), Eur. J. Immunol., 24, 2640]. Arteriosclerotic lesions of are often associated with lymphocyte infiltration, suggesting a possibility that the Fas ligand of T cells, together with the Fas of macrophages, is responsible for controlling arteriosclerosis [c.f. Kenji Harada (1997), Gendai Iryou, 29, 109].

#### Myocarditis and cardiomyopathy

The Fas/Fas ligand system is likely to be involved in the pathogeneses of autoimmune heart diseases, such as ischemic heart disease, viral heart disease, dilated cardiomyopathy and chronic cardiomyopathy. Myocarditis is inflammation of the heart muscle considered to be caused mainly by viruses, such as coxsackie virus, and is typified by chest pain, arrhythmia, heart failure or shock, after cold-like symptoms. Cardiomyopathy is defined as "a disease of the cardiac muscle of unknown cause," although its cause is also considered likely to be as a result of viral infection. In studies of mouse myocarditis models, with heart failure, apoptotic cells (as evidenced by condensation and/or fragmentation of the nuclei), are observed in the mouse heart after viral inoculation. Increase in Fas expression is also observed in the mouse heart, which has led to speculation that the condition was as a result of apoptosis induced by Fas ligand derived from infiltrating inflammatory cells, predominantly lymphocytes [c.f. Takehiko Yamada, et al. Gendai Iryou , (1997), 29, 119]. It is known that apoptosis is induced in cultured rat cardiac muscle cells by ischemia, concurrently with an increase in mRNA coding for Fas in the cells [c.f. Tanaka M., et al.,

(1994), Circ. Res. 75, 426].

#### Renal diseases

In many chronic renal diseases, reconstitution of the tissue within the glomeruli results in the accumulation of extracellular substrates within the glomeruli, thereby promoting sclerosis of the glomeruli, leading to the pathological loss of filtering function and, ultimately, to chronic renal failure. In a model of progressive glomerulosclerosis, the sclerotic regions exhibited typical apoptotic appearances, at electron microscopic levels, and an increase in apoptosis in glomeruli is observed, consistent with a decrease in the number of glomerular cells associated with the progression of sclerosis [c.f. Sugiyama H., et al., (1996), Kidney Int., 49, 103]. In acute glomerular nephritis, it is known that the disease is alleviated by the apoptotic reduction in numbers of abnormally proliferated mesangial cells [c.f. Shimizu A., et al., (1995), Kidney Int., 47, 114 and Baker A. J., et al., (1994), J. Clin. Invest., 94, 2105]. In diseases such as purpura nephritis and lupus nephritis, a marked increase in cells expressing Fas in glomeruli has been reported [c.f. Takemura T., et al., (1995), Kidney Int., 48, 1886].

#### Hypoplastic anemia

On the surface of hematopoietic precursor cells of patients with hypoplastic anemia, Fas expression is remarkably elevated compared with that in normal individuals, suggesting the involvement of Fas in the decrease of hematopoietic stem cells in these patients [c.f. Maciejewski, J. P., et al., (1995), Br. J. Haematol., 91, 245].

#### Hepatitis

In fulminant hepatitis, it is known that apoptosis is induced in many hepatocytes. Extensive hepatocyte death, similar to that

observed in fulminant hepatitis, is observed upon intraperitoneal administration of the anti-Fas antibody Jo2 to mice. Thus, it is considered likely that the pathogenesis of fulminant hepatitis involves Fas-induced apoptosis of hepatocytes [c.f. Kamogawa, Y., et al., (1996), Molecular Medicine, 33, 1284; and Ogasawara, J., et al., (1993), Nature, 364, 806]. In immunohistochemical studies, enhanced Fas expression was observed in the cytoplasm of hepatocytes in the regions showing high levels of hepatocyte necrosis, such as within lesions of chronic hepatitis and on the cell membrane of hepatocytes of lesions of hepatic diseases, such as fatty liver [c.f. Hiramatsu, N., et al., (1994), Hepatology, 19, 1354 and Takatani, M., et al., (1996), International Hepatology Commun., 4, 334].

In addition, Fas is expressed in the lesions of chronic persistent hepatitis C and chronic active hepatitis, both of which show dispersed staining of hepatocytes surrounded by infiltrating lymphocytes, which are apparently cytotoxic T cells [c.f. Mita, E., et al., (1994), Biochem. Biophys. Res. Commun., 204, 468]. Cytotoxic T cells have the function of inducing apoptosis in infected cells via the Fas ligand expressed on their surface. They similarly induce apoptosis in the normal cells located near-by. This affect on local, normal cells, called the bystander disorder, results from the fact that many cells in the body express Fas after either their own infection or after infection of neighboring cells. In chronic hepatitis cases, where hepatitis C virus-derived RNA is substantially reduced after the administration of interferon, Fas expression in the hepatic tissue decreases markedly.

Hepatocytes of patients with acute hepatic failure have increased amounts of Fas on the cell surface, and undergo apoptosis when exposed to apoptosis-inducing, anti-Fas antibodies. Further, in alcoholic hepatitis, Fas ligand is

expressed on the hepatocytes themselves within the pseudoacinus [c.f. Galle, P. R., et al., (1995), J. Exp. Med., 182, 1223]. In studies involving *in situ* hybridization of Fas ligand gene expression, expression was found, both in hepatic infiltrating lymphocytes, in cases of acute hepatic failure, and also in the hepatocytes themselves in the pseudoacinus in cases of alcoholic cirrhosis (as above). Thus, it is speculated that apoptosis is induced by different mechanisms in viral cirrhosis and alcoholic cirrhosis, but in both cases the Fas/Fas ligand system is abnormal. In a mouse model of hepatitis, it is known that hepatic disorder is inhibited by the administration of a substance capable of inhibiting the binding of Fas ligand to Fas [c.f. Kondo, T., et al., (1997), Nature Medicine, 3, 409].

#### Acquired immunodeficiency syndrome

Immunodeficiency in patients infected with the human immunodeficiency virus (HIV) results, at least partially, from the apoptotic cell deaths of numerous immune cells not infected with HIV. Helper T cells die on contact with HIV. Since growth factor from helper T cells is essential for the suppression of apoptosis in cytotoxic T cells, depletion of helper T cells results in the apoptosis of cytotoxic T cells. It is also considered likely that apoptosis of immune cells in HIV-infected patients is due to abnormalities of the Fas/Fas ligand system, based on observations that expression of Fas in the peripheral blood lymphocytes of HIV-infected patients correlates well with the pathological progression of the disease [c.f. Dhein, J., et al., (1995), Behring Inst. Mitt., 96, 13 and McCloskey, T. W., et al., (1995), Cytometry, 22, 111]. Fas-positive peripheral blood lymphocytes from non-infected individuals do not readily undergo apoptosis by Fas stimulation, whereas peripheral blood lymphocytes from infected patients undergo Fas-induced apoptosis within a short period [c.f. Owen-Schaub, L. B., et al., (1992), Cell Immunol., 140, 197].

Rejection after organ transplantation

Rejection after organ transplantation shares certain similarities with autoimmune diseases, except that the transplanted organ is being attacked by cytotoxic T cells from a donor. Thus, alleviation of the symptoms can be expected if the functions of cytotoxic T cells can be suppressed.

For the diseases listed above, effective means for their treatment is by the elimination of abnormal cells (e.g., autoreactive T cells in autoimmune diseases, foam cells in arteriosclerosis, mesangial cells in acute glomerular nephritis, infected cells in viral infections, and synovial cells in rheumatoid arthritis) and/or by the protection of normal tissues or cells.

The problem lies in the fact that agents which are only capable of inducing Fas-mediated apoptosis, are highly likely to cause disorders in normal tissues, even though abnormal cells are eliminated. On the other hand, agents only capable of inhibiting Fas-mediated apoptosis cannot eliminate abnormal cells, even though they may be able to protect normal cells. For example, the anti-mouse Fas monoclonal antibody Jo2 has apoptosis-inducing activity but causes fulminant hepatitis in mice [c.f. Ogasawara, J., et al., (1993), *Nature*, 364, 806-809].

To date, an anti-Fas antibody which can be used in the treatment and/or prophylaxis of any of the above diseases, but which is not associated with any undesirable side effects, is not known.

Immunoglobulin G (IgG) is composed of two light polypeptide chains (L chains), each having a molecular weight of about 23,000 kD, and two heavy polypeptide chains (H chains), each

having a molecular weight of about 50,000 kD. Both H and L chains consist of a repeated region of conserved amino acids consisting of about 110 residues. This region is referred to herein as a "domain", and constitutes the basic three-dimensional structural unit of the IgG. The H and L chains consist of four and two consecutive domains, respectively.

When antibody amino acid sequences are compared, the amino-terminal domain of both H and L chains is found to be more variable than the other domains. It is, therefore, referred to as the 'variable' domain (V domain). The V domains of H and L chains associate with each other by their complementary nature to form variable regions in the amino-termini of IgG molecules. The other domains associate to form constant regions. The constant region sequences are characteristic for a given species. For example, the constant regions of mouse IgG differ from those of human IgG, and a mouse IgG molecule is recognized as a foreign protein by the human immune system. Administration of a mouse IgG molecule into a human subject results in the production of a human anti-mouse antibody (hereinafter referred to as "HAMA") response [Schroff et al., (1985), Cancer Res., 45, 879-885]. Accordingly, a mouse antibody cannot be repeatedly administered to a human subject. For effective administration, the antibody must be modified to avoid inducing the HAMA response, while maintaining the antibody specificity.

Data from X-ray crystallography analysis indicates that the immunoglobulin fold generally forms a long cylindrical structure comprising two layers of antiparallel  $\beta$ -sheets, each consisting of three or four  $\beta$ -chains. In a variable region, three loops from each of the V domains of H and L chains cluster together to form an antigen-binding site. Each of these loops is termed a complementarity determining region (CDR). The CDR's have the highest variability in amino acid sequence. The portions of the

variable region that are not part of a CDR are called "framework regions" ("FR" regions) and generally play a role in maintaining the structure of CDR's.

Kabat and co-workers compared the primary sequences of a number of variable regions of H and L chains and identified putative CDR's or framework regions, based on sequence conservation (E. A. Kabat et al., Sequences of immunological interest, 5th edition, NIH Publication, No. 91-3242). Further, they classified the framework regions into several subgroups which share common amino acid sequences. They also identified framework regions that correspond between mouse and human sequences.

Studies on the structural characteristics of IgG molecules have led to the development of methods for preparing humanized antibodies, which do not provoke a HAMA response, as described below.

Initial suggestions were directed towards the preparation of a chimaeric antibody, by joining the variable region of a mouse antibody to the constant regions of human origin [Morrison, S. L., et al., (1984), Proc. Natl. Acad. Sci. USA, 81, p6851-6855]. Such a chimeric antibody, however, still contains many non-human amino acid residues, and thus can cause a HAMA response, especially when administered for a prolonged period [Begent et al., (1990), Br. J. Cancer, 62, p487 et seq.].

The grafting of CDR segments alone into a human antibody was then proposed, in order to further reduce the number of non-human amino acid sequences causing the HAMA response [Jones, P. T. et al., (1986), Nature, 321, 522-525]. However, the grafting of the CDR portions alone was generally found to be insufficient to maintain the activity of the immunoglobulin against an antigen.

Based on data from X-ray crystallography, Chothia and co-workers [Chothia et al., (1987), J. Mol. Biol., 196, 901-917] determined that:

- 1) A CDR has a region involved in antigen binding and a region involved in maintaining the structure of the CDR itself. Possible three-dimensional structures for CDR's can be classified into several classes with characteristic patterns (canonical structures); and
- 2) The classes of canonical structures are determined not only by the CDR sequences but also by the nature of amino acids in specific positions in the framework regions.

As a result, it has been suggested that the CDR-grafting technique should also involve the grafting of certain amino acid residues from the framework regions into the human antibody backbone [Queen et al., International Patent Publication No. WO90/07861].

In the context of the above, an antibody from a non-human mammal from which the CDR's are obtained for grafting is hereinafter termed a 'donor' molecule. A human antibody into which the CDR's are grafted is hereinafter termed an 'acceptor' molecule.

In performing CDR-grafting, the structures of the CDR region should ideally be conserved and the activity of the immunoglobulin molecule should be maintained. The following factors may, therefore, be relevant:

- 1) the subgroup of the acceptor; and
- 2) the nature of the amino acid residues that are transferred from the framework regions of the donor.

WO90/07861] proposed a method for deciding whether an amino acid residue from the donor FR was to be grafted along with the CDR sequence. According to this method, an amino acid residue from a FR region is grafted onto the acceptor, together with the CDR sequence, if the residue meets at least one of the following criteria:

- 1) The amino acid in the human framework region of the acceptor is rarely found at that position in the acceptor, whereas the corresponding amino acid in the donor is commonly found at that position in the acceptor
- 2) the amino acid is closely located to one of the CDR's; and
- 3) the amino acid has a side-chain atom within approximately 3 Å of a CDR, as judged by a three-dimensional model of the immunoglobulin, and is potentially able to interact with an antigen or a CDR of a humanized antibody.

However, no one has successfully obtained a humanized, anti-Fas, IgG type antibody which has apoptosis-inducing activity.

#### Objects of the Invention

It is an object of the present invention to provide an anti-Fas antibody, or similar molecule, which can be evaluated in an animal model of a human Fas-related disease condition.

It is a further object of the present invention to provide a humanized anti-Fas antibody, or similar molecule, useful in the treatment and/or prophylaxis of conditions arising from abnormalities in the Fas/Fas ligand system.

It is a further object of the present invention to provide a humanized antibody having apoptosis-inducing activity.

Monoclonal antibodies that specifically bind to human Fas and which also have apoptosis-inducing activity are known, but none of them is capable of binding mouse Fas. Likewise, monoclonal antibodies that bind mouse Fas are known, but none binds human Fas. Thus, so far, it has not been possible to

establish a mouse model to evaluate the pharmaceutical efficacy of anti-human Fas monoclonal antibodies.

Summary of the Invention

Thus, in a first aspect, the present invention provides a molecule having a binding region specific for a Fas epitope, the epitope being conserved between a primate and a non-primate animal.

In a second aspect, the present invention provides a molecule having a binding region specific for a common, mammalian, Fas epitope.

The present invention further provides an antibody as produced by the hybridoma HFE7A having the accession number FERM BP-5828, as well as a molecule having at least six antibody CDR's, the antibody being specific for human Fas, wherein the CDR's have identity with the CDR's of the antibody as produced by the hybridoma HFE7A having the accession number FERM BP-5828.

Thus, in a further aspect, the present invention provides a humanized anti-Fas antibody capable of  
a) inducing apoptosis in abnormal cells, especially as described above, by binding Fas antigen on the cell surface, and  
b) preventing apoptosis in normal cells which would otherwise be induced as a result of the binding of Fas ligand to Fas antigen.

More preferably, the present invention provides an anti-Fas antibody comprising one or more heavy chain heavy chain subunits substantially having an amino acid sequence selected from the group consisting of:

the amino acid sequence 1 to 451 of SEQ ID No. 143;  
the amino acid sequence 1 to 451 of SEQ ID No. 145;  
the amino acid sequence 1 to 451 of SEQ ID No. 147; and  
the amino acid sequence 1 to 451 of SEQ ID No. 157  
of the Sequence Listing.

More preferably, the antibody has one or more light chain subunits substantially having an amino acid sequence selected from the group consisting of:

the amino acid sequence 1 to 218 of SEQ ID No. 107;  
the amino acid sequence 1 to 218 of SEQ ID No. 127;  
the amino acid sequence 1 to 218 of SEQ ID No. 129; and  
the amino acid sequence 1 to 218 of SEQ ID No. 131  
of the Sequence Listing.

Of the above preferred antibodies, a preferred group are those wherein the heavy chain consists essentially of the amino acid sequence 1 to 451 of SEQ ID No. 157 of the Sequence Listing, it being then further preferred that the light chain consists essentially of the amino acid sequence 1 to 218 of SEQ ID No. 107 of the Sequence Listing.

Another preferred category are those antibodies wherein one or more light chain subunits substantially have an amino acid sequence selected from the group consisting of:  
the amino acid sequence 1 to 218 of SEQ ID No. 127;  
the amino acid sequence 1 to 218 of SEQ ID No. 129; and  
the amino acid sequence 1 to 218 of SEQ ID No. 131  
of the Sequence Listing, and one or more heavy chain heavy chain subunits substantially have an amino acid sequence selected from the group consisting of:  
the amino acid sequence 1 to 451 of SEQ ID No. 143;  
the amino acid sequence 1 to 451 of SEQ ID No. 145; and  
the amino acid sequence 1 to 451 of SEQ ID No. 147;  
of the Sequence Listing.

It will be appreciated that the term "substantially" is used herein to refer to the possibility of altering the sequences referred to. Such alteration may be by substitution, deletion, insertion or inversion, for example, but will generally be restricted in scope, in order to retain the characteristics of the sequence in question.

In an alternative aspect, the present invention provides a humanized anti-Fas antibody which is capable of binding both human and mouse Fas antigen.

There is further provided an agent for the prophylaxis and/or treatment of conditions involving a Fas disorder, comprising an anti-Fas antibody, or molecules similar thereto, as active ingredient.

Methods of treatment of conditions involving Fas disorders are further provided, such methods involving the administration of non-toxic, or substantially non-toxic, doses of antibodies of the invention to an animal, especially a human, in need thereof.

Other objects, aims, aspects and embodiments of the present invention will become apparent hereinbelow.

#### Brief Description of the Drawings

Figure 1 is a diagram depicting the construction of phFas-AIC2.

Figure 2 is a diagram depicting the construction of pME-H and pME-L.

Figure 3 is a figure showing the results of ELISA for the determination of the epitope recognized by the HFE7A antibody.

Figure 4 is a figure showing the results of a competitive assay for the determination of the epitope recognized by the HFE7A antibody.

Figure 5 is a figure showing the results of toxicity testing of HFE7A.

Figure 6 is a figure showing the results of testing with a fulminant hepatitis model.

Figure 7 is a figure showing the results of testing of prevention of collagen-induced arthritis.

Figure 8 is a summary of the first step PCR for the production of VHH-DNA.

Figure 9 is a summary of the second step PCR for the production of VHH-DNA.

Figure 10 is a summary of the third step PCR for the production of VHH-DNA.

Figure 11 is a summary of the construction of the expression plasmid carrying VHH-DNA fragment.

Figure 12 is a summary of the first step PCR for the production of VHM-DNA.

Figure 13 is a summary of the second step PCR for the production of VHM-DNA.

Figure 14 is a summary of the construction of the expression plasmid carrying VHM-DNA fragment.

Figure 15 is a summary of the first step PCR for the production of VMM-DNA.

Figure 16 is a summary of the second step PCR for the production of VMM-DNA.

Figure 17 is a summary of the third step PCR for the production of VMM-DNA.

Figure 18 is a summary of the construction of the expression plasmid carrying VMM-DNA fragment.

Figure 19 shows the positions to which the light chain sequencing primers bind.

Figure 20 is a summary of the first step PCR for the production of VD-DNA.

Figure 21 is a summary of the second step PCR for the production of VD-DNA.

Figure 22 is a summary of the third step PCR for the production of VD-DNA.

Figure 23 is a summary of the construction of the expression plasmid carrying VD-DNA fragment.

Figure 24 is a summary of the construction of the DNA (IG5'-DNA) fragment comprising CH1 region of human IgG1 and an intron.

Figure 25 is a summary of the construction of the genomic DNA (IG3'-DNA) fragment comprising hinge region, CH2 region, CH3 region and introns of human IgG1.

Figure 26 is a summary of the construction of the expression

plasmid pEg7AH-H.

Figure 27 shows the positions to which the heavy chain sequencing primers bind.

Figure 28 is a graph depicting the binding activity of the humanized anti-Fas antibodies to the human Fas fusion protein.

Figure 29 shows competitive inhibition of HFE7A and the humanized anti-Fas antibodies for the human Fas fusion protein.

Figure 30 shows the cytotoxicity of the humanized HFE7A to WR19L12a.

Figure 31 shows the outline of the first stage PCR for the preparation of LPDHH-DNA.

Figure 32 shows the outline of the second stage PCR for the production of LPDHH-DNA.

Figure 33 shows the outline of the third stage PCR for the production of LPDHH-DNA.

Figure 34 shows the outline of the construction of an expression plasmid carrying the LPDHH-DNA fragment.

Figure 35 shows the outline of the first stage PCR for the preparation of LPDHM-DNA.

Figure 36 shows the outline of the second stage PCR for the production of PDHM-DNA.

Figure 37 shows the outline of the third stage PCR for the preparation of LPDHM-DNA.

Figure 38 shows the outline of the construction of a plasmid carrying the LPDHM-DNA fragment.

Figure 39 shows the outline of the first stage PCR for the preparation of HPD1.2-DNA.

Figure 40 shows the outline of the second stage PCR for the preparation of HPD1.2-DNA.

Figure 41 shows the construction of a plasmid carrying the HPD1.2-DNA fragment.

Figure 42 shows where primers for sequencing pEgPDHV3-21 bind.

Figure 43 shows the construction of high-level expression vectors for the humanized light chains.

Figure 44 shows the construction of high-level expression vectors for humanized heavy chains.

Figure 45 shows the binding activity for the human Fas fusion protein for the supernatants of Example 12.

Figure 46 shows the results of competitive inhibition of HFE7A antibody by the supernatants of Example 12.

Figure 47 shows the results of inducing apoptosis in T cells by culture supernatant fluids of Example 12.

Figure 48 shows a comparison of FR-amino acid sequences of HFE7A, each human acceptor and light chain of each humanized antibody;

Figure 49 shows a comparison of FR-amino acid sequences of HFE7A, each human acceptor and heavy chain of each humanized antibody;

Figure 50 is a summary of the first step PCR for the production of LEU1-DNA;

Figure 51 is a summary of the second step PCR for the production of LEU1-DNA;

Figure 52 is a summary of the first step PCR for the production of LEU2-DNA;

Figure 53 is a summary of the second step PCR for the production of LEU2-DNA;

Figure 54 is a summary of the first step PCR for the production of LEU3-DNA;

Figure 55 is a summary of the second step PCR for the production of LEU3-DNA;

Figure 56 shows the outline of the construction of expression plasmid carrying DNA encoding Eu type humanized light chain;

Figure 57 is a summary of the first step PCR for the production of HEU1HA-DNA;

Figure 58 is a summary of the second step PCR for the production of HEU1HA-DNA;

Figure 59 is a summary of the first step PCR for the production of HEU2HA-DNA;

Figure 60 is a summary of the second step PCR for the production of HEU2HA-DNA;

Figure 61 is a summary of the first step PCR for the production of HEU3HA-DNA;

Figure 62 is a summary of the second step PCR for the production of HEU3HA-DNA;

Figure 63 shows the outline of the construction of expression plasmid carrying DNA encoding Eu type humanized heavy chain;

Figure 64 shows the binding activity of the human Fas fusion protein for the supernatants of culture of transformed COS-7 cells;

Figure 65 shows the results of competitive inhibition of HFE7A antibody by the supernatants of culture of transformed COS-7 cells;

Figure 66 shows the results of inducing apoptosis in WR19L12a by culture supernatant fluid of culture of transformed COS-7 cells;

Figure 67 is a summary of the first step PCR for the production of HHHV-DNA;

Figure 68 is a summary of the second step PCR for the production of HHHV-DNA;

Figure 69 shows the outline of the construction of plasmid carrying HHHV-DNA;

Figure 70 shows the position to which sequencing primers for the HHH type humanized heavy chain is bound;

Figure 71 shows the outline of the construction of expression plasmid carrying HHHV-DNA;

Figure 72 shows the binding activity in the human Fas fusion protein for the supernatants of culture of transformed COS-1 cells; and

Figure 73 shows the results of competitive inhibition of binding of human Fas fusion protein with HFE7A antibody by the supernatants of cultures of transformed COS-1 cells.

Detailed description of the Invention

A particular advantage of the antibodies of the present invention (including the HFE7A antibody) is that they are not only able to induce apoptosis in abnormal cells expressing Fas, but that they are also able to inhibit apoptosis in normal cells.

No known monoclonal antibody which binds human Fas and which has apoptosis-inducing activity is capable of binding mouse Fas. Monoclonal antibodies that bind mouse Fas are known, but none of them binds human Fas. Thus, no known anti-Fas antibody can be evaluated in disease model mice. By contrast, the antibodies of the present invention (including the HFE7A antibodies) are able to be evaluated in disease mice models, thereby both providing means for ensuring pharmaceutical efficacy and also establishing a model for the investigation of the role of Fas, in general.

It is believed that the advantages of the antibodies of the present invention arise from their ability to recognize a conserved epitope on the Fas antigen. Fas is a common molecule, but varies from species to species. Without being bound by theory, it is believed that there is at least one conserved region of Fas, which is common to all mammals, and which is necessary for the Fas apoptosis-inducing function. The molecules of the present invention recognize a conserved Fas epitope. In this respect, when comparing murine and human Fas, for example, the epitope in question need not necessarily be absolutely identical in the two molecules, provided that the epitope binding region of the molecule is able to recognize both. However, in general, the epitope will be exactly the same.

Many antibodies directed against Fas are known, including those capable of inducing apoptosis, but none has previously been obtained which bound any kind of consensus sequence. The antibodies of the present invention, by way of contrast, do bind a consensus sequence. Accordingly, as an extension of the theory, instead of merely acting to incapacitate or interfere by generalized binding to Fas, which can have dangerous and unpredictable effects, such as with Jo2 and fulminant hepatitis in mice (*supra*), the antibodies of the present invention actually act at the Fas active site, thereby mimicking a natural ligand, rather than merely non-specifically binding the Fas antigen.

If a normal laboratory mouse, such as a BALB/c mouse, is immunized with human Fas, cells producing antibodies which bind both human Fas and mouse Fas will be eliminated in the thymus, in the usual course of eliminating autoreactive antibodies. Thus, in order to obtain a mouse monoclonal antibody which is directed to an epitope conserved between human and mouse and which, accordingly, binds both human Fas and mouse Fas, it is necessary to use a mouse in which such elimination has been partially or completely disabled.

It has been speculated that the Fas/Fas ligand system is involved in this elimination process of auto-reactive T cells in the thymus [*c.f.* Shin Yonehara (1994) *Nikkei Science Bessatsu*, 110, 66-77]. Therefore, by immunizing a mouse having a mutation in the Fas/Fas ligand system (such a mouse is hereinafter referred to as a "Fas knock-out mouse" or "Fas/Fas ligand deficient mouse"), for example, one that is unable to express the gene coding for Fas, antibodies which bind mouse Fas as well as human Fas can be obtained.

Antibodies against Fas may generally be obtained by administering an immunogenically effective amount of a substance comprising an immunogenic epitope of heterologous Fas to a non-human animal, which is at least partially deficient in the apoptotic elimination of autoreactive T cells, and selecting antibodies from the animal thereafter.

The substance carrying the Fas epitope may be Fas itself, or may be another suitable substance, such as a fusion protein.

Selection of appropriate antibodies is within the skill of those in the art, and is exemplified below. In particular, it is preferred to use the immunized animal of the method of the invention to obtain at least one monoclonal antibody, which is readily obtainable using methods well known in the art.

It will also be appreciated that, for ease of manipulation, it is preferred that the non-human animal is a mouse, although other rodent species, such as rabbits, and other mammals, in general, such as goats and macaques, may also be used, although such systems are not quite so well characterised as the mouse. It will also be appreciated that it is preferred that the Fas used for administration be human, although, if desired, antibodies of the invention may be obtained for other mammals. However, it is generally envisaged that, owing to the sharing of a common epitope, the antibodies of the present invention have universal application.

Using the above method, a hybridoma was prepared which produces a novel anti-Fas monoclonal antibody binding both human and mouse Fas. A Fas knock-out mouse was immunized with human Fas and then the spleen cells were fused with mouse myeloma cells, and monoclonal antibodies were then purified from the culture supernatant.

The novel anti-Fas monoclonal antibodies of the present invention induced apoptosis in T-cells of mice and other non-human primates which express Fas. Thus, the present invention demonstrates that there is a common epitope, at least in primate (including human) and rodent (at least murine) Fas, which can be recognized by the antibody of the present invention, and which is able to induce apoptosis when the antibody of the present invention binds thereto.

The novel anti-Fas monoclonal antibody thus recovered has proved to be efficacious in alleviating the severity of the symptoms of autoimmune disease model mice. Moreover, it has been demonstrated that this anti-Fas monoclonal antibody does not induce hepatic disorders, which has previously been a problem.

The respective genes for both chains of the new antibody were also cloned and sequenced, in order to obtain the amino acid sequences of the CDR's. Expression vectors, comprising the respective genes for the heavy and light chains, were constructed in order to produce a recombinant anti-Fas antibody.

These recombinant antibodies, obtained in culture supernatant fluids of animal cells co-transfected with these vectors, was demonstrated to react with Fas.

The anti-Fas antibodies thus obtained, and their recombinant antibody clones, are able to protect the liver from Fas-induced fulminant hepatitis, and are also effective in the prevention and treatment of rheumatoid arthritis.

Accordingly, it has now been demonstrated that it is readily possible to provide antibodies which are able both to induce apoptosis via Fas in abnormal cells and to inhibit Fas-induced apoptosis in normal cells, and are, therefore, effective in the prevention, treatment and/or prophylaxis of diseases attributable to abnormalities of the Fas/Fas ligand system.

The method for obtaining the antibodies of the invention involved grafting of the CDR amino acid sequences of the mouse-derived anti-Fas monoclonal antibodies into a human antibody. Recombinant antibodies, which were not immunogenic to human subjects, but still had Fas-binding activity, were successfully obtained.

The present invention allows the construction of humanized antibodies which have a minimal risk of inducing a HAMA response, whilst still having an effective antibody effector function.

Homology of a binding region, or epitope, refers to the sequence of the region. Indeed, in general, amino acid sequences surrounding an epitope are often conserved, in different animals, for antigens bound by a common monoclonal antibody which recognizes homologous proteins. Homology of the amino acid sequence in the region is high in many cases. The epitope to which the antibody of the present invention is bound is not limited to such a region. Homology in the primary structure is not conserved but, rather, homology in a higher-order structure is conserved. Thus, epitopes which are conserved between primates and non-primates includes reference to regions having high homology in the higher-order structure of the proteins, particularly those that can be recognized by one monoclonal antibody.

As used herein, the terms "human" and "humanized", in relation to antibodies, relate to any antibody which is expected to elicit little, or no, immunogenic response in a human subject, the subject in question being an individual or a group.

It will be appreciated that, in general, it is preferred that all of the CDR's from a given antibody be grafted into an acceptor antibody, in order to preserve the binding region for the Fas epitope, or epitope binding region, as it is generally referred to herein. However, there may be occasions when it is appropriate or desirable for less than the total amount of CDR's to be grafted into the donor, and these are envisaged by the present invention. It will also be understood that grafting generally entails the replacement, residue for residue, of one amino acid or region, for another. However, occasionally, especially with the transfer of a region, one or more residues may be added or omitted, as desired, and that such deletions and insertions, as well as appropriate replacements and inversions, are within the skill of those in the art.

The epitope binding region of the present invention is a region of the molecule which corresponds to an epitope binding site of an antibody. The epitope binding region need not be derived directly from any particular antibody, or pair of

antibodies, and may not resemble any particular epitope binding region. The only requirement is that the epitope binding region resemble the recognition site of an antibody insofar as it is able to bind an antigen, in this case, a Fas epitope. Even though the epitope binding region may be designed using the CDR's from a known antibody, if these are then grafted into a human antibody, the resulting epitope binding region may not necessarily resemble that from the known antibody, although a large degree of similarity is desirable, from the point of view of maintaining binding specificity.

We particularly prefer that all of the CDR's from the non-human antibody be grafted into the human antibody. Further, we prefer that certain areas of the framework regions be incorporated into the acceptor antibody (also referred to as the human antibody, herein) in order to maintain the 3-dimensional structure of the non-human binding site. Such areas of the framework regions typically comprise individual amino acid residues selected for their importance (significant residues), in accordance with the guidelines below. In particular, those residues which are rare in human, but common in the relevant non-human antibody, and those residues having a high probability of interacting directly with the epitope or the recognition site, are preferred to be grafted together with the CDR's.

When grafting the CDR's into the human antibody, it will normally be the case that the non-human CDR replaces a relevant human CDR in its entirety, particularly where both are of the same length. However, it may also be the case that only a part of a human CDR is replaced, or only a part of the non-human CDR is grafted, the two usually going hand-in-hand.

It will also be appreciated that the CDR's from the non-human antibody should generally be used to replace the

corresponding CDR's in the human antibody. In the situation where a skeleton human light or heavy chain is used, which only has positions for insertion of CDR's, rather than actually having CDR's, then similar considerations apply.

It will also be understood that the human heavy and light chains need not necessarily come from the same human antibody, nor even from the same class. What is important is that the sequence of the selected donor matches, as closely as possible, the sequence of the non-human antibody. The importance of matching the two chains (light/light or heavy/heavy) is that the resulting antibody should have a epitope binding region as closely resembling that of the original non-human antibody as possible, to ensure the best binding. Thus, the present invention also envisages the possibility of using matches which are not the closest possible, where there is a reasonable expectation that the resulting recombinant antibody will serve the required purpose.

The molecules of the present invention are preferably antibodies, although this is not necessary, provided that the epitope binding region binds a Fas epitope. Thus, isolated and stabilized binding sites, for example, may be attached to an affinity purification column support, or an administration method may comprise an adjuvant carrier molecule, for example, to which are attached epitope binding regions of the invention. For ease of reference, the molecules of the present invention will generally be termed antibodies herein, but such reference encompasses all molecules of the invention, unless otherwise indicated.

Where the molecule of the invention is an antibody, it will be appreciated that any appropriate antibody type may be emulated, or employed, such as IgG, IgA, IgE and IgM, with IgG

being generally preferred.

Where molecules and antibodies are discussed herein, it will also be understood that similar considerations apply, *mutatis mutandis*, to any nucleic acid sequences encoding them, as appropriate.

Certain preferred embodiments of the present invention are as follows.

It is preferred that the antibody of the invention binds a peptide comprising the amino acid sequence of SEQ ID No. 1 of the Sequence Listing.

The antibody is preferably IgG and, more preferably, comprises a light chain polypeptide protein selected individually from the amino acid sequence 1 to 218 of SEQ ID No. 50, the amino acid sequence 1 to 218 of SEQ ID No. 52, the amino acid sequence 1 to 218 of SEQ ID No. 54, the amino acid sequence 1 to 218 of SEQ ID No. 107, and the amino acid sequence 1 to 218 of SEQ ID No. 109 of the Sequence Listing, and wherein the heavy chain polypeptide protein preferably comprises the amino acid sequence 1 to 451 of SEQ ID No. 89 or the amino acid sequence 1 to 451 of SEQ ID No. 117 of the Sequence Listing.

An antibody of the invention, in a preferred embodiment, has a light chain and a heavy chain, the heavy chain having the following general formula (I):

-FRH<sub>1</sub>-CDRH<sub>1</sub>-FRH<sub>2</sub>-CDRH<sub>2</sub>-FRH<sub>3</sub>-CDRH<sub>3</sub>-FRH<sub>4</sub>- (I)

wherein FRH<sub>1</sub> represents any amino acid sequence consisting of 18 to 30 amino acids, CDRH<sub>1</sub> represents the sequence as defined in SEQ ID No. 2 of the Sequence Listing, FRH<sub>2</sub> represents any amino acid sequence consisting of 14 amino acids, CDRH<sub>2</sub> represents the sequence as defined in SEQ ID No. 3 of the

Sequence Listing, FRH<sub>3</sub> represents any amino acid sequence consisting of 32 amino acids, CDRH<sub>3</sub> represents the sequence as defined in SEQ ID No. 4 of the Sequence Listing, FRH<sub>4</sub> represents any amino acid sequence consisting of 11 amino acids, and each amino acid binds another via a peptide bond, and the light chain having the following general formula (II):

-FRL<sub>1</sub>-CDRL<sub>1</sub>-FRL<sub>2</sub>-CDRL<sub>2</sub>-FRL<sub>3</sub>-CDRL<sub>3</sub>-FRL<sub>4</sub>- (II)

wherein FRL<sub>1</sub> represents any amino acid sequence consisting of 23 amino acids, CDRL<sub>1</sub> represents the sequence as defined in SEQ ID No. 5 of the Sequence Listing, FRL<sub>2</sub> represents any amino acid sequence consisting of 15 amino acids, CDRL<sub>2</sub> represents the sequence as defined in SEQ ID No. 6 of the Sequence Listing, FRL<sub>3</sub> represents any amino acid sequence consisting of 32 amino acids, CDRL<sub>3</sub> represents the sequence as defined in SEQ ID No. 7 of the Sequence Listing, FRL<sub>4</sub> represents any amino acid sequence consisting of 10 amino acids, and each amino acid binds another via a peptide bond.

The invention also provides DNA and RNA encoding any one of the light or heavy chain polypeptide proteins described above. More preferred is DNA comprising the nucleotide sequence 100 to 753 of SEQ ID No. 49, DNA comprising the nucleotide sequence 100 to 753 of SEQ ID No. 51, DNA comprising the nucleotide sequence 100 to 753 of SEQ ID No. 53 and/or DNA comprising the nucleotide sequence 84 to 2042 of SEQ ID No. 88 of the Sequence Listing.

DNA encoding the antibodies of the present invention is also provided, as are recombinant DNA vectors comprising such DNA, and host cells transformed with such vectors. The host is preferably transformed with a separate vector for each heavy and light chain encoded, so will usually contain two vectors, although the present invention also envisages a host transformed with only one expression vector encoding all sequences to be expressed. Such a host cell is preferably mammalian.

Each of the following transformed strains incorporate particularly preferred plasmids of the present invention and are each preferred: (light chains) *E. coli* pHSGMM6 SANK73697 (FERM BP-6071), *E. coli* pHSGHM17 SANK73597 (FERM BP-6072), *E. coli* pHSGHH7 SANK73497 (FERM BP-6073), *E. coli* pHSHM2 SANK 70198 and *E. coli* pHSHH5 SANK 70398 (FERM BP-6272); (heavy chains) *E. coli* pgHSL7A62 (FERM BP-6274) SANK73397 (FERM BP-6074) and *E. coli* pgHPDHV3 SANK 70298 (FERM BP-6273).

The present invention also provides a method for producing a humanized anti-Fas antibody comprising culturing the above host cells, and then recovering the humanized anti-Fas antibody from the culture.

Further provided is an agent for the prophylaxis or treatment of diseases attributable to abnormalities of the Fas/Fas ligand system comprising as an active ingredient the antibody of the present invention, especially where the diseases are as defined above. Targeted diseases are autoimmune diseases (systemic lupus erythematosus, Hashimoto disease, rheumatoid arthritis, graft versus host disease, Sjögren syndrome, pernicious anemia, Addison's disease, scleroderma, Goodpasture syndrome, Crohn's disease, autoimmune hemolytic anemia, sterility, myasthenia gravis, multiple sclerosis, Basedow disease, thrombopenia purpura, or insulin dependent diabetes mellitus). Separate preparations are also envisaged for: allergy; rheumatoid arthritis; arteriosclerosis; myocarditis or cardiomyopathy; glomerular nephritis; hypoplastic anemia; hepatitis (fulminant hepatitis, chronic hepatitis, viral hepatitis (hepatitis C, hepatitis B, hepatitis D) or alcoholic hepatitis); and rejection after organ transplantation.

The framework regions (FR's) are present in the variable region of an H or L chain subunit of an immunoglobulin molecule. For instance, FRH<sub>1</sub> refers to the framework region located at the most N-terminal position

in the variable region of an H chain subunit, and FRL<sub>4</sub> refers to the fourth framework region from the N-terminus of the variable region of an L chain subunit. Similarly, CDRH<sub>1</sub>, for example, refers to the CDR present at the most N-terminal position in the variable region of an H chain subunit, and CDRL<sub>3</sub> refers to the third CDR from the N-terminus of the variable region of an L chain subunit. The FRs flank the CDR regions in any light or heavy chain.

It will be appreciated that the antibodies of the present invention can be obtained by, for example, grafting each CDR of the L chain and H chain subunit of the anti-Fas monoclonal antibody HFE7A into a corresponding CDR region of a human antibody, thereby humanizing it.

In one embodiment, an anti-Fas monoclonal antibody, suitable to prepare a humanized anti-Fas antibody according to the present invention, may be obtained by culturing a suitable hybridoma which, in turn, may be obtained by immunizing a Fas knock-out mouse with human Fas and subsequently fusing the spleen cells from the mouse with mouse myeloma cells.

Preparation of a monoclonal antibody typically involves the following steps:

- a) purification of a biomacromolecule for use as the immunizing antigen;
- b) preparation of antibody producing cells, after first immunizing an appropriate animal using injections of the antigen, bleeding the animal and assaying the antibody titer, in order to determine when to remove the spleen;
- c) preparation of myeloma cells;
- d) fusing the antibody producing cells and myeloma cells;
- e) selecting a hybridoma producing an antibody of interest;
- f) preparing a single cell clone (cloning);
- g) optionally, culturing the hybridoma cells, or growing animals into which the hybridoma cells have been transplanted, for large scale preparation of the monoclonal antibody; and
- h) testing the biological activities and the specificity, or assaying marker agent properties, of the monoclonal antibody thus prepared.

The general procedure followed for the preparation of an anti-Fas monoclonal antibody is herein below described in more detail, in line with the above described steps. However, it will be appreciated that the method described below only represents one way of preparing a suitable antibody, and other procedures may be followed, as desired, such as for instance, using cells other antibody producing cells than spleen cells and other cell lines than myeloma.

a) Preparation of antigen

A recombinant protein (hereinafter referred to as "recombinant human Fas"), effective as the Fas antigen, can be obtained by transfecting the monkey cell line COS-1 with the expression vector pME18S-mFas-AIC, which encodes a fusion protein comprising the extracellular domain of human Fas and the extracellular domain of the mouse interleukin-3 receptor [IL3R - c.f. Nishimura, Y., et al., (1995), J. Immunol., 154, 4395-4403], and collecting and partially purifying the expression product. The plasmid pHFas-AIC2 was constructed by inserting DNA encoding a human Fas and mouse IL3R fusion protein into pME18S, which is an expression vector for animal cells. As noted above, the materials used, such as the DNA encoding Fas, the vector and the host, are not restricted to those mentioned.

The resulting human Fas and IL3R fusion protein, referred to herein as recombinant human Fas, collected from the culture supernatant of the transformed COS-1 cells may be partially purified by a suitable method, such as ion-exchange chromatography using a Resource Q column (tradename; Pharmacia).

As a suitable alternative, purified Fas obtained from the cell membranes of human cell lines can be used as the antigen. Further, since the primary structure of Fas is known [c.f. Itoh,

N., et al., (1991), Cell, 66, 233-243], a peptide comprising a suitable portion of the amino acid sequence of human Fas, such as that of SEQ ID No. 1 of the Sequence Listing, may be chemically synthesized by any suitable method and used as the antigen.

b) Preparation of antibody producing cells

An experimental animal is immunized with the immunogen produced in step a), suitably mixed with an adjuvant, such as Freund's complete, or incomplete, adjuvant and alum. In the present instance, a suitable experimental animal is a Fas knock-out mouse, which may be produced by the method of Senju et al. [Senju, S., et al., (1996), International Immunology, 8, 423].

Suitable administration routes to immunize the mouse include the subcutaneous, intraperitoneal, intravenous, intradermal and intramuscular injection routes, with subcutaneous and intraperitoneal injections being preferred.

Immunization can be by a single dose or, more preferably, by several repeated doses at appropriate intervals (preferably 1 to 5 weeks). Immunized mice are monitored for anti-Fas antibody activity in their sera, and an animal with a sufficiently high antibody titer is selected as the source of antibody producing cells. Selecting an animal with a high titer makes the subsequent process more efficient. Cells for the subsequent fusion are generally harvested from the animal 3 to 5 days after the final immunization.

Methods for assaying antibody titer include various well known techniques such as radioimmunoassay (RIA), solid-phase enzyme immunoassay (ELISA), fluorescent antibody assay and passive hemagglutination assay, with RIA and ELISA preferred for reasons of detection sensitivity, rapidity, accuracy and potential for automation.

Determination of antibody titer may be performed, for example, by ELISA, as follows. First, purified or partially purified Fas is adsorbed onto the surface of a solid phase, such as a 96-well ELISA plate, followed by blocking any remaining surface, to which Fas has not bound, with a protein unrelated to the antigen, such as bovine serum albumin (BSA). After washing, the well surfaces are contacted with serially diluted samples of the antibody preparations to be tested (for example, mouse serum) to enable binding of the anti-Fas antibody in the samples to the antigen. An enzyme-labelled, anti-mouse antibody, as the secondary antibody, is added to bind the mouse antibody. After washing, the substrate for the enzyme is added, and anti-Fas binding activity can then be assayed by determining a suitable change, such as absorbance change due to color development.

c) Preparation of myeloma cells

In general, cells from established mouse cell lines serve as the source of myeloma cells. Suitable cell lines include: 8-azaguanine resistant mouse (derived from BALB/c) myeloma strains, P3X63Ag8U.1 (P3-U1) [Yelton, D. E., et al., Current Topics in Microbiology and Immunology, 81, 1-7, (1978)], P3/NSI/1-Ag4-1(NS-1) [Kohler, G., et al., European J. Immunology, 6, 511-519 (1976)], Sp2/O-Ag14(SP-2) [Shulman, M., et al., Nature, 276, 269-270 (1978)], P3X63Ag8.653 (653) [Kearney, J. F., et al., J. Immunology, 123, 1548-1550 (1979)] and P3X63Ag8 (X63) [Horibata, K. and Harris, A. W., Nature, 256, 495-497 (1975)]. The cell line selected is serially transferred into an appropriate medium, such as 8-azaguanine medium [RPMI-1640 medium supplemented with glutamine, 2-mercaptoethanol, gentamicin, fetal calf serum (FCS), and 8-azaguanine], Iscove's Modified Dulbecco's Medium (IMDM) or Dulbecco's Modified Eagle Medium (DMEM). The cells are then transferred to a normal medium, such as ASF104 medium (Ajinomoto, K. K.) containing 10% w/v FCS, 3 to 4 days

prior to fusion, in order to ensure that at least  $2 \times 10^7$  cells are available on the day of fusion.

d) Cell fusion

The antibody producing cells used in fusion are plasma cells and their precursor cells, lymphocytes, which may be obtained from any suitable part of the animal. Typical areas are the spleen, lymph nodes, peripheral blood, or any appropriate combination thereof, spleen cells most commonly being used.

After the last booster injection, tissue in which antibody producing cells are present, such as the spleen, is removed from a mouse having the predetermined antibody titer to prepare antibody producing cells. The currently favored technique for fusion of the spleen cells with the myeloma cells prepared in step c), employs polyethylene glycol, which has relatively low cytotoxicity and the fusion procedure using it is simple. An example of this technique is as follows.

The spleen and myeloma cells are washed well with serum-free medium (such as RPMI 1640) or phosphate buffered saline (PBS), and then mixed, so that the number ratio of spleen cells to myeloma cells is approximately between 5 : 1 and 10 : 1, and then centrifuged. After the supernatant has been discarded and the pelleted cells sufficiently loosened, a suitable amount, generally 1 ml, of serum-free medium containing 50% (w/v) polyethylene glycol (m.w. 1,000 to 4,000) is added dropwise with mixing. Subsequently, 10 ml of serum-free medium is slowly added and then the mixture centrifuged. The supernatant is discarded again, and the pelleted cells are suspended in an appropriate amount of HAT medium [a solution of hypoxanthin, aminopterin and thymidine (these three compounds, together, are also known as "HAT") and mouse interleukin-2 (IL-2)]. The suspension is then dispensed into the wells of culture plates (also referred herein

simply as "plates") and incubated in the presence of 5% v/v CO<sub>2</sub> at 37°C for about 2 weeks, with the supplementary addition of HAT medium as appropriate.

e) Selection of hybridomas

When the myeloma strain used is resistant to 8-azaguanine, i.e., it is deficient in the hypoxanthin guanine phosphoribosyl transferase (HGPRT) enzyme, any unfused myeloma cells and any myeloma-myeloma fusions are unable to survive in HAT medium. On the other hand, fusions of antibody producing cells with each other, as well as hybridomas of antibody producing cells with myeloma cells can survive, the former only having a limited life. Accordingly, continued incubation in HAT medium results in selection of only the desired hybridomas.

The resulting hybridomas are then grown up into colonies in HAT medium lacking aminopterin (HT medium). Thereafter, aliquots of the culture supernatant are removed to determine anti-Fas antibody titer by, for example, ELISA. When the above recombinant human Fas fusion protein is used as the ELISA antigen, it is also necessary to eliminate clones producing an antibody which specifically binds the extracellular domain of the mouse IL3 receptor. The presence or absence of such a clone may be verified, for example, by ELISA using mouse IL3 receptor, or its extracellular domain, as the antigen.

Although the above selection procedure is exemplified using an 8-azaguanine resistant cell line is used, it will be appreciated that other cell lines may be used with appropriate selection markers and with appropriate modifications to the media used.

f) Cloning

Hybridomas which have been shown to produce anti-Fas

specific antibodies, using a method similar to that described in the step b) to determine antibody titer, are then transferred to another plate for cloning. Suitable cloning methods include: the limiting dilution method, in which hybridomas are diluted to contain 1 cell per well of a plate and then cultured; the soft agar method, in which colonies are recovered after culturing in soft agar medium; using a micromanipulator to separate a single cell for culture; and "sort-a-clone", in which single cells are separated by a cell sorter. Limiting dilution is generally the most simple and is commonly used.

Whichever cloning procedure is selected is repeated 2 to 4 times for each well demonstrating an antibody titer, and clones having stable antibody titers are selected as anti-Fas monoclonal antibody producing hybridomas. Hybridomas producing an anti mouse Fas antibody are selected by a similar method to obtain an anti-Fas monoclonal antibody producing cell line. A suitable mouse Fas useful for this purpose, for example, is the fusion protein expressed by cultured animal cells transfected with the expression vector pME18S-mFas-AIC. This plasmid has DNA encoding a fusion protein comprising the extracellular domain of mouse Fas and the extracellular domain of the mouse IL3 receptor [c.f. Nishimura, Y., et al., (1995), J. Immunol., 154, 4395-4403, incorporated herein by reference]. Other sources of murine Fas include purified mouse Fas and cells which expressing mouse Fas on their surface.

The mouse-mouse hybridoma HFE7A was selected by the above methodology. Its specific preparation is described in the accompanying Examples. HFE7A is a cell line producing an anti-Fas monoclonal antibody suitable as the base in preparing a humanized anti-Fas antibody of the present invention, and was deposited with the Kogyo Gijutsuin Seimeい-Kogaku Kogyo Gijutsu Kenkyujo on February 19, 1997, in accordance with the Budapest

Treaty on the Deposition of Microorganisms, and was accorded the accession number FERM BP-5828. Accordingly, when preparing an antibody using the mouse-mouse hybridoma HFE7A, the preparation may be performed by following a procedure starting from step g) below, with steps a) to f), above, omitted.

g) Culture of hybridoma to prepare monoclonal antibody

The hybridoma obtained by the preceding steps is then cultured in normal medium, rather than HT medium. Large-scale culture can be performed by roller bottle culture, using large culture bottles, or by spinner culture. The supernatant from the large-scale culture is then harvested and purified by a suitable method, such as gel filtration, which is well known to those skilled in the art, to obtain an anti-Fas monoclonal antibody. The hybridoma may also be grown intraperitoneally in a syngeneic mouse, such as a BALB/c mouse or a Nu/Nu mouse, to obtain ascitic fluid containing an anti-Fas monoclonal antibody in large quantities. Commercially available monoclonal antibody purification kits (for example, MAbTrap GII Kit; Pharmacia) may conveniently be used to purify the harvested antibodies.

Monoclonal antibodies prepared as above, and which have been selected for specificity for human and mouse Fas, have a high specificity to human and mouse Fas.

h) Assay of monoclonal antibody

Determination of the isotype and the subclass of the monoclonal antibody thus obtained may be performed as follows. Suitable identification methods include the Ouchterlony method, ELISA and RIA. The Ouchterlony method is simple, but requires concentration of the solutions used when the concentration of the monoclonal antibody is low. By contrast, when ELISA or RIA is used, the culture supernatant can be reacted directly with an antigen adsorbed on a solid phase and with secondary antibodies

having specificities for the various immunoglobulin isotypes and subclasses to identify the isotype and subclass of the monoclonal antibody. However, in general, it is preferred to use a commercial kit for identification, such as a Mouse Typer Kit (tradename; BioRad).

Quantification of protein may be performed by the Folin-Lowry method, for example, or by calculation based on the absorbance at 280 nm [1.4 (OD<sub>280</sub>) = Immunoglobulin 1 mg/ml].

Identification of the Fas epitope that the monoclonal antibody recognizes may be performed as follows. First, various partial Fas structures are prepared. The partial structures may be prepared synthetically, such as by oligopeptide synthesis, or *in vivo* by using a suitable host, such as *E. coli*, which has been transformed by a suitable vector incorporating DNA encoding the desired fragments. Both methods are frequently used in combination for the identification of the epitope recognized by the epitope binding region. For example, a series of polypeptides having appropriately reduced lengths, working from the C- or N-terminus of the antigen protein, can be prepared by genetic engineering techniques well known to those skilled in the art. By establishing which fragments react with the antibody, an approximate idea of the epitopic site can be obtained.

The epitope can be more closely identified by synthesizing a variety of smaller oligopeptides corresponding to portions or mutants of the peptide, or peptides, recognized by the antibody. Oligopeptide synthesis is generally used for the preparation of these smaller fragments. Identification of the epitope may then be established by binding studies or by competitive inhibition studies with the recombinant human Fas fusion protein in ELISA, for example. Commercially available kits, such as the SPOTs Kit (Genosys Biotechnologies, Inc.) and a series of multipin peptide

synthesis kits based on the multipin synthesis method (Chiron Corp.) may be conveniently used to obtain a large variety of oligopeptides.

DNA encoding the heavy and light chains of the anti-Fas monoclonal antibody prepared above may be obtained by preparing mRNA from hybridoma cells producing the anti-Fas monoclonal antibody, converting the mRNA into cDNA by reverse transcription, and then isolating the DNA encoding the heavy and or light chains of the antibody, respectively. This DNA may then be used to generate the humanized anti-Fas antibody of the present invention.

Extraction of mRNA can be performed by the guanidinium thiocyanate-hot phenol method or by the guanidinium thiocyanate-guanidinium HCl method, for example, but the guanidinium thiocyanate-cesium chloride method is preferred. Preparation of mRNA from cells is generally performed by first preparing total RNA and then purifying mRNA from the total RNA by using a poly(A)<sup>+</sup> RNA purification matrix, such as oligo(dT) cellulose and oligo(dT) latex beads. Alternatively, mRNA may be prepared directly from a cell lysate using such a matrix. Methods for preparing total RNA include: alkaline sucrose density gradient centrifugation [c.f. Dougherty, W. G. and Hiebert, E., (1980), *Virology*, 101, 466-474]; the guanidinium thiocyanate-phenol method; the guanidinium thiocyanate-trifluoro cesium method; and the phenol-SDS method. The currently preferred method uses guanidinium thiocyanate and cesium chloride [c.f. Chirgwin, J. M., et al., (1979), *Biochemistry*, 18, 5294-5299].

The thus obtained poly(A)<sup>+</sup> RNA can be used as the template in a reverse transcriptase reaction to prepare single-strand cDNA [(ss) cDNA]. The (ss) cDNA obtained by the use of reverse transcriptase, as described above, can then be converted to

double stranded (ds) cDNA. Suitable methods for obtaining the ds cDNA include the S1 nuclease method [c.f. Efstratiadis, A., et al., (1976), *Cell*, 7, 279-288], the Gubler-Hoffman method [c.f. Gubler, U. and Hoffman, B. J., (1983), *Gene*, 25, 263-269] and the Okayama-Berg method [c.f. Okayama, H. and Berg, P., (1982), *Mol. Cell. Biol.*, 2, 161-170]. However, the currently preferred method involves the polymerase chain reaction [PCR - c.f. Saiki, R. K., et al., (1988), *Science*, 239, 487-491, incorporated herein by reference] using single-strand cDNA as the template. Thus the preferred procedure is labelled "RT-PCR", as it involves reverse transcription and PCR.

The ds cDNA obtained above may then be integrated into a cloning vector and the resulting recombinant vector can then be used to transform a suitable micro-organism, such as *E. coli*. The transformant can be selected using a standard method, such as by selecting for tetracycline resistance or ampicillin resistance encoded by the recombinant vector. If *E. coli* is used, then transformation may be effected by the Hanahan method [c.f. Hanahan, D., (1983), *J. Mol. Biol.*, 166, 557-580, incorporated herein by reference]. Alternatively, the recombinant vector may be introduced into competent cells prepared by co-exposure to calcium chloride and either magnesium chloride or rubidium chloride. If a plasmid is used as a vector, then it is highly desirable that the plasmid harbours a drug-resistant gene, such as mentioned above, in order to facilitate selection. Brute force selection is possible, but not preferred. Although plasmids have been discussed, it will be appreciated that other cloning vehicles, such as lambda phages, may be used.

To select transformants for those which carry cDNA encoding a subunit of an anti-human Fas antibody of interest, various methods, such as those described below, can be used. When the cDNA of interest is specifically amplified by RT-PCR, these steps

may be omitted.

(1) Screening by polymerase chain reaction

If all or part of the amino acid sequence of the desired protein has been elucidated, then sense and antisense oligonucleotide primers corresponding to separate non-contiguous parts of the amino acid sequence can be synthesised. These primers can then be used in the polymerase chain reaction technique [c.f. Saiki, R. K., et al. (1988), Science, 239, 487-491] to amplify the desired DNA fragment coding for the mouse anti-human Fas monoclonal antibody subunit. The template DNA used in the PCR may be, for example, cDNA synthesized by reverse transcription from mRNA of the hybridoma producing the anti-human Fas monoclonal antibody HFE7A (FERM BP-5828).

The DNA fragment thus synthesised may either be directly integrated into a plasmid vector, such as by using a commercial kit, or may be labelled with, for example,  $^{32}\text{P}$ ,  $^{35}\text{S}$  or biotin, and then used as a probe for colony hybridization or plaque hybridization to obtain the desired clone.

Harvesting of DNA encoding each subunit of anti-human Fas monoclonal antibody from the appropriate transformants obtained above may be performed by well known techniques, such as those described by Maniatis, T., et al. [in "Molecular Cloning A Laboratory Manual" Cold Spring Harbor Laboratory, NY, (1982) , incorporated herein by reference]. For example, the region of DNA coding for the desired subunit may be excised from plasmid DNA after separating the fraction corresponding to the vector DNA from a transformant which has been determined to possess the necessary plasmid.

(2) Screening using a synthetic oligonucleotide probe

If all or part of the amino acid sequence of the desired

protein has been elucidated, then a short contiguous sequence, which is also representative of the desired protein, may be used to construct an oligonucleotide probe. The probe encodes the amino acid sequence but, owing to the degeneracy of the genetic code, there may be a large number of probes that can be prepared. Thus, an amino acid sequence will normally be selected which can only be encoded by a limited number of oligonucleotides. The number of oligonucleotides which it is necessary to produce can be further reduced by the substitution of inosine where any of the four normal bases can be used. The probe is then suitably labelled, such as with  $^{32}\text{P}$ ,  $^{35}\text{S}$  or biotin, and is then hybridized with denatured, transformed DNA from the transformant which has been immobilised on a nitrocellulose filter. Positive strains show up by detection of the label on the probe.

Wherever appropriate, DNA sequences may be sequenced by various well known methods in the art including, for example, the Maxam-Gilbert chemical modification technique [c.f. Maxam, A. M. and Gilbert, W. (1980) in "Methods in Enzymology" 65, 499-276] and the dideoxy chain termination method using M13 phage [c.f. Messing, J. and Vieira, J. (1982), Gene, 19, 269-276]. In recent years, a further method for sequencing DNA has gained wide acceptance, and involves the use of a fluorogenic dye in place of the conventional radioisotope in the dideoxy method. The whole process is computerised, including the reading of the nucleotide sequence after electrophoresis. Suitable machinery for the process is, for example, the Perkin-Elmer Sequence robot "CATALYST 800" and the Perkin-Elmer model 373A DNA Sequencer. The use of this technique renders the determination of DNA nucleotide sequences both efficient and safe.

By using techniques such as those described above, determination of the DNA sequence can be performed efficiently and safely. Based on the data of the thus determined respective

nucleotide sequences of the DNA of the present invention and the respective N-terminal amino acid sequences of the heavy and light chains, the entire amino acid sequences of the heavy and light chains of a monoclonal antibody of the present invention can be determined.

For example, the HFE7A monoclonal antibody of the present invention, which is suitable to provide CDR's for grafting into a humanized antibody of the present invention, is an immunoglobulin G1 (IgG1) molecule and is, thus, a complex composed of  $\gamma_1$  heavy chain and  $\kappa$  light chain subunits. Preferred methods for determining partial amino acid sequences of these respective subunits include, for example, isolating the respective subunits by a suitable technique, such as electrophoresis or column chromatography, and then analyzing the N-terminal amino acid sequences of the respective subunits using, for example, an automated protein sequencer (for example, PPSQ-10, Shimadzu Seisakusyo, K. K.).

The heavy and light chains of an immunoglobulin each consist of a variable region and a constant region, the variable region of each chain further consisting of three CDR's and four framework regions flanking the CDR's.

The amino acid sequence of the constant region is constant within any given subclass, regardless of the antigen recognized. On the other hand, the amino acid sequence of the variable region, at least for the CDR's, is specific for each antibody. However, it has been established by comparison studies, using data on amino acid sequences of numerous antibodies, that both the locations of CDR's and the lengths of framework sequences are roughly similar among antibody subunits belonging to the same subgroup [c.f. Kabat, E. A., et al., (1991), in "Sequences of Proteins of Immunological Interest Vol. II," U.S.

Department of Health and Human Services, incorporated herein by reference]. Therefore, by comparing the amino acid sequences of the heavy and light chains of the anti-Fas monoclonal antibody HFE7A with those known amino acid sequence data, for example, the CDR's and the framework regions, as well as the location of the constant region, in each of the amino acid sequences determined above, can be established.

The length of FRH<sub>1</sub>, i.e., the most N-terminal framework region of heavy chains, has been occasionally found to be shorter than the normal length of 30 amino acids. For example, the shortest known FRH<sub>1</sub> in mouse IgG1, of the same subtype as HFE7A, is only 18 amino acids [c.f. Kabat et al., *ibid.*). Accordingly, in the antibody of the present invention, it will be appreciated that the length of that part of the overall molecule corresponding to FRH<sub>1</sub> may be of appropriate length, typically between 18 and 30 amino acids, but preferably about 30 amino acids, provided that the necessary Fas binding activity is not lost. In fact, we have established that activity can be retained, even without grafting the FR into the humanized antibody.

The three-dimensional structure of the Fas binding region is mainly determined by the sequences in the variable regions, with support being provided by the constant regions. The framework regions provide structure to the CDR's which are chemically and structurally configured to interact with the antigen.

Accordingly, an existing antibody, or a portion thereof, which recognizes an antigen other than Fas can be selected and modified to recognize Fas by suitable alteration of the CDR's, in accordance with the guidelines above (see, for example, U.S.

patent No. 5,331,573). In order to

conserve as much binding activity as possible, it is generally preferred to select acceptor chains which have the greatest similarity to the donor chains. Such modified peptides thus modified are useful in the present invention, such as in prevention or treatment of diseases

attributable to abnormalities of the Fas/Fas ligand system.

Construction of a mutant wherein one or more amino acids in an amino acid sequence is deleted may be performed, for example, by cassette mutagenesis (c.f. Toshimitsu Kishimoto, "Shin-Seikagaku Jikken Kouza 2: Kakusan III Kumikae DNA Gijutsu," 242-251).

DNA sequences may be prepared by any appropriate method, and many are known. A suitable method, especially for shorter sequences, is chemical synthesis using a conventional method, such as the phosphite triester method [c.f. Hunkapiller, M., et al., (1984), *Nature*, 310, 105-111]. Selection of codons for any amino acid may be from any of the recognized codons corresponding to a desired amino acid, and such selection may be arbitrary, or by taking into account frequency of a given codon in a host, or because it is possible to create a restriction site by appropriate selection, without changing the amino acid sequence, for example. Partial modification of the nucleotide sequence can be accomplished by site specific mutagenesis utilizing synthetic oligonucleotide primers coding for the desired modifications [c.f. Mark, D. F., et al., (1984), *Proc. Natl. Acad. Sci. USA*, 81, 5662-5666], by conventional techniques.

Hybridization of DNA with DNA encoding the heavy or light chain of an anti-Fas monoclonal antibody of the present invention can be determined, for example, by using an appropriate fragment of DNA of the invention labelled with ( $\alpha$ -<sup>32</sup>P)dCTP, for example, as a probe by a method such as the random primer method [c.f. Feinberg, A. P. and Vogelstein, B. (1983), *Anal. Biochem.*, 132, 6-13] or by the nick translation method [c.f. Maniatis, T., et al., (1982), in "Molecular Cloning A Laboratory Manual" Cold Spring Harbor Laboratory, NY]. A suitable technique is as follows.

First, the potentially hybridizing DNA is adsorbed onto a nitrocellulose or nylon membrane, for example, being subjected to alkaline treatment if necessary, and then being fixed by heating or UV irradiation. In a preferred method, the membrane is next immersed in prehybridization solution containing  $6 \times$  SSC ( $1 \times$  SSC is an aqueous solution of 0.15 M NaCl and 0.015 M citric acid tri-sodium), 5% v/v Denhardt solution and 0.1% v/v sodium dodecyl sulfate (SDS), and incubated at 55°C for 4 hours or more. Then, the probe previously prepared is dissolved in similar prehybridization solution to a final specific activity of  $1 \times 10^6$  cpm/ml, followed by incubation at 60°C overnight. Subsequently, the membrane is washed at room temperature by repeated washing with  $6 \times$  SSC for 5 minutes and further with  $2 \times$  SSC for 20 minutes, and is then subjected to autoradiography.

By using such a method, DNA hybridizable with the DNA coding for the heavy or light chain of an anti-Fas monoclonal antibody which can serve as the basis for a humanized anti-Fas antibody of the present invention is isolatable from any cDNA library or genomic library [c.f. Maniatis, T., et al., (1982), in "Molecular Cloning A Laboratory Manual" Cold Spring Harbor Laboratory, NY]. Such DNA is comprised within the scope of the present invention, the essential features of the hybridization being  $6x$  SSC and 55°C, preferably 60°C and more preferably 70°C.

Integration of DNA of the present invention thus obtained into an expression vector allows transformation of prokaryotic or eukaryotic host cells. Such expression vectors will typically contain suitable promoters, replication sites and sequences involved in gene expression, thereby allowing the DNA to be expressed in the host cell.

Suitable prokaryotic host cells include, for example, *E.*

*coli* (*Escherichia coli*) and *Bacillus subtilis*. In order to express the gene of interest in such host cells, these host cells may be transformed with a plasmid vector containing a replicon derived from a species compatible with the host, typically having an origin of replication and a promoter sequence, such as lac UV5. These vectors preferably have sequences capable of conferring a selection phenotype on the transformed cell.

A suitable strain of *E. coli* is strain JM109 derived from *E. coli* K12. Suitable vectors include pBR322 and the pUC series plasmids. Suitable promoters include the lactose promoter (lac), the tryptophan lactose promoter (trc), the tryptophan (trp) promoter, the lipoprotein (lpp) promoter, the lambda ( $\lambda$ ) PL promoter derived from bacteriophage  $\lambda$ , and the polypeptide chain elongation factor Tu (tufB) promoter. In general, it will be appreciated that the present invention is not limited to the use of such hosts, vectors, promoters, etc., as exemplified herein and that any suitable systems may be used, as desired.

A suitable preferred strain of *Bacillus subtilis* is strain 207-25, and a preferred vector is pTUB228 [c.f. Ohmura, K., et al., (1984), J. Biochem., 95, 87-93]. A suitable promoter is the regulatory sequence of the *Bacillus subtilis*  $\alpha$ -amylase gene. If desired, the DNA sequence encoding the signal peptide sequence of  $\alpha$ -amylase may be linked to the DNA of the present invention to enable extracellular secretion.

Eukaryotic hosts include cell hosts from vertebrate and yeast species. An example of vertebrate cells used is the monkey COS-1 cell line [c.f. Gluzman, Y., (1981), Cell, 23, 175-182]. Suitable yeast cell hosts include baker's yeast (*Saccharomyces cerevisiae*), methylotrophic yeast (*Pichia pastoris*) and fission yeast (*Schizosaccharomyces pombe*). It will be appreciated that other hosts may also be used as desired.

In general, the requirements for suitable expression vectors for vertebrate cells are that they comprise: a promoter, usually upstream of the gene to be expressed; an RNA splicing site; a polyadenylation site; and a transcription termination sequence, as well as any other functionalities required, such as an origin of replication. A suitable plasmid is pSV2dhfr containing the SV40 early promoter [c.f. Subramani, S., et. al, (1981), Mol. Cell. Biol., 1, 854-884], but many others are known to those skilled in the art.

Suitable eukaryotic micro-organisms are the yeasts, such as *S. cerevisiae*, and suitable expression vectors for yeasts include pAH301, pAH82 and YEp51. Suitable vectors contain, for example, the promoter of the alcohol dehydrogenase gene [c.f. Bennetzen, J. L. and Hall, B. D., (1982), J. Biol. Chem., 257, 3018-3025] or of the carboxypeptidase Y GAL10 promoter [c.f. Ichikawa, K., et. al, (1993), Biosci. Biotech. Biochem., 57, 1686-1690]. If desired, the DNA sequence encoding the signal peptide sequence of carboxypeptidase Y may be linked, for example, to the DNA to be expressed in order to enable extracellular secretion.

When COS cells are used as hosts, vectors suitably comprise the SV40 replication origin, enabling autonomous replication, a transcription promoter, a transcription termination signal and an RNA splicing site. The expression vectors can be used to transform the cells by any suitable method, such as the DEAE-dextran method [c.f. Luthman, H, and Magnusson, G. (1983), Nucleic Acids Res., 11, 1295-1308], the phosphate calcium-DNA co-precipitation method [c.f. Graham, F. L. and Van der Eb, A. J., (1973), Virology, 52, 456-457] and the electric pulse electroporation method [c.f. Neumann, E., et. al., (1982), EMBO J., 1, 841-845].

In a preferred embodiment, COS cells are co-transfected with two separate expression vectors - one containing DNA encoding a protein comprising at least the variable region of the heavy chain of the HFE7A antibody, preferably as part of a whole humanized heavy chain, and one containing DNA encoding a protein comprising at least the variable region of the light chain of the HFE7A antibody, preferably as part of a whole humanized light chain, these vectors being expressed simultaneously to generate a humanized recombinant anti-human Fas antibody.

Transformants of the present invention may be cultured using conventional methods, the desired proteins being expressed either intra- or extra-cellularly. Suitable culture media include various commonly used media, and will generally be selected according to the host chosen. For example, suitable media for COS cells include RPMI-1640 and Dulbecco's Modified Eagle Minimum Essential medium (DMEM) which can be supplemented with, as desired, fetal bovine serum (FBS).

The culture temperature may be any suitable temperature which does not markedly depress the protein synthesis capability of the cell, and is preferably in the range of 32 to 42°C, most preferably 37°C, especially for mammalian cells. If desired, culture may be effected in an atmosphere containing 1 to 10% (v/v) carbon dioxide.

The transformant strains *E. coli* pME-H and *E. coli* pME-L, each transformed with a recombinant DNA vector for the expression in animal cells of DNA encoding the heavy and light chains, respectively, of an anti-Fas monoclonal antibody useful to prepare humanized anti-Fas antibodies of the present invention, were deposited with the Kogyo Gijutsuin Seimeい-Kogaku Kogyo Gijutsu Kenkyujo on March 12, 1997 in accordance with the Budapest Treaty, and the accession numbers FERM BP-5868 and BP-

5867, respectively, were accorded them. Therefore, by transforming cultured animal cells such as COS-1 with the recombinant vectors isolated from the deposited strains and culturing the transformant cells, a recombinant anti-Fas antibody can be produced in culture.

The protein expressed by the transformants of the present invention may be isolated and purified by various well known methods of separation according whether the protein is expressed intra- or extra- cellularly and depending on such considerations as the physical and chemical properties of the protein. Suitable specific methods of separation include: treatment with commonly used precipitating agents for protein; various methods of chromatography such as ultrafiltration, molecular sieve chromatography (gel filtration), adsorption chromatography, ion exchange chromatography, affinity chromatography and high performance liquid chromatography (HPLC); dialysis; and combinations thereof.

By the use of such methods as described above, the desired protein can be readily obtained in high yields and high purity.

In order to optimally humanize, in this instance, a mouse anti-Fas monoclonal antibody, it is preferred to graft the variable regions into a human antibody, at least so that the whole of each CDR is incorporated into the human antibody, and preferably also so that significant residues of the FR sequences are grafted into the human antibody in order to maintain as much of the structure of the binding site as possible. This may be accomplished by any one of the following three methods:

- 1) using heavy and light chains from the same known human antibody; or
- 2) using heavy and light chains derived from different human antibodies, which have high sequence homology to, or share

consensus sequences with, the chains of the donor, while at the same time maintaining the combination of the subgroups of the acceptor chains; or

3) selecting the FR's of heavy and light chains that have the highest homologies with the FR's of the donor from a library of the primary sequences of human antibodies, regardless of the combination of the subgroups.

Such a selection method based upon sequence homology alone, with no other constraints, makes it possible for the donor and the acceptor to share at least 70% amino acid identity in the FR portions. By adopting this approach, it is possible to reduce the number of amino acids grafted from the donor, with respect to known methods, and thus to minimise induction of the HAMA response.

The term 'amino acid sequence homology', as used herein, refers to the similarity of amino acid sequence between two different polypeptides or proteins. Amino acid sequence homology can be assessed by any one of a number of methods, commonly involving the computerised search of sequence databases. These methods are well known to the person skilled in the art. We prefer that the homology is assessed over the length of the framework regions.

It will be appreciated that the role of amino acid residues that occur rarely in the donor subgroup cannot be fully defined, since techniques for predicting the three-dimensional structure of an antibody molecule from its primary sequence (hereinafter referred to as "molecular modelling") have limited accuracy. Known methods, such as the method of Queen and co-workers (Queen *et al.*, *supra*), do not indicate whether the amino acid residue from the donor or from the acceptor should be selected in such a position. The selection of an acceptor molecule based upon

sequence homology alone can significantly reduce the need to make this type of selection.

In the present invention, various humanized antibodies are constructed. Each uses, as a starting point, the HFE7A antibody as a donor. However, the exact nature of the residues transferred to the acceptor molecule and the nature of the acceptor molecule is varied in each case.

In construction of the humanized antibody in which the human monoclonal antibody Eu is used as an acceptor, the above-mentioned method 1) is used, with the FR's being transferred.

In addition, we have discovered a further refinement to this method by the provision of an additional selection procedure, designed to identify amino acids from the donor FR's which are important in the maintenance of the structure and function of the donor CDR regions.

Once the human acceptor molecule has been selected for a given chain, then selection of the amino acid residues to be grafted from a FR of a donor is carried out as follows.

The amino acid sequences of the donor and the acceptor are aligned. If the aligned amino acid residues of the FRs differ at any position, it is necessary to decide which residue should be selected. The residue that is chosen should not interfere with, or only have a minimal effect upon, the three-dimensional structure of the CDRs derived from the donor.

Queen et al. [International Patent Publication No. WO90/07861, incorporated herein by reference] proposed a method for deciding whether an amino acid residue from the donor FR was to be grafted along with the CDR sequence. According to this method, an amino acid residue from a FR region is grafted onto

the acceptor, together with the CDR sequence, if the residue meets at least one of the following criteria:

- 1) The amino acid in the human framework region of the acceptor is rarely found at that position in the acceptor, whereas the corresponding amino acid in the donor is commonly found at that position in the acceptor;
- 2) the amino acid is closely located to one of the CDR's; and
- 3) the amino acid has a side-chain atom within approximately 3 Å of a CDR, as judged by a three-dimensional model of the immunoglobulin, and is potentially able to interact with an antigen or a CDR of a humanized antibody.

A residue identified by criterion (2), above, often displays the characteristics of criterion (3). Thus, in the present invention, criterion (2) is omitted and two new criteria are introduced. Accordingly, in the present invention, where an amino acid residue is grafted from a donor FR along with the CDR, it should meet at least one of the following criteria:

- a) the amino acid in the human framework region of the acceptor is rarely found at that position in the acceptor, whereas the corresponding amino acid in the donor is commonly found at that position in the acceptor;
- b) the amino acid has a side-chain atom within approximately 3 Å of a CDR, as judged by a three-dimensional model of the immunoglobulin, and is potentially able to interact with an antigen or a CDR of a humanized antibody;
- c) the amino acid is found in a position which is involved in determining the structure of the canonical class of the CDR;
- d) the position of the amino acid is found at the contact surface of the heavy and light chains.

With respect to criterion (a), an amino acid is defined as "common" when it is found at that position in 90 % or more of the antibodies of the same subclass [Kabat et al., *supra*]. An

amino acid is defined as "rare" when it is found in less than 10% of antibodies of the same subclass.

With respect to criterion (c), the position of a canonical class determinant residues can be determined unambiguously according to the information provided by Chothia and co-workers [Chothia et al., *supra*].

With respect to criteria (b) and (d), it is necessary to carry out molecular modeling of the variable regions of the antibody in advance. While any commercially available software for molecular modeling can be used, we prefer that the AbM software is used [Oxford Molecular Limited, Inc.].

Predictions made by molecular modeling have limited accuracy. Therefore, in the present invention, the structure prediction obtained by molecular modeling was assessed by comparing it with X-ray crystallography data from the variable regions of various antibodies.

When using a structural model generated by molecular modeling (such as AbM software), two atoms are presumed to be in contact with each other by Van der Waal's forces when the distance between the two atoms is less than the sum of their Van der Waal's radii plus 0.5 Å. A hydrogen bond is presumed to be present when the distance between polar atoms, such as an amide nitrogen and a carbonyl oxygen of the main and side chains, is shorter than 2.9 Å (the average length for a hydrogen bond) plus 0.5 Å. Furthermore, when the distance between the two oppositely charged atoms is shorter than 2.85 Å plus 0.5 Å, they are presumed to form an ion pair.

The positions of amino acids in the FR which frequently contact a CDR were identified, based upon X-ray crystallography

data from the variable regions of various antibodies. These positions were determined irrespective of subgroups. For the light chains, these are positions 1, 2, 3, 4, 5, 23, 35, 36, 46, 48, 49, 58, 69, 71 and 88, and for the heavy chains positions 2, 4, 27, 28, 29, 30, 36, 38, 46, 47, 48, 49, 66, 67, 69, 71, 73, 78, 92, 93, 94 and 103. The above amino acid numbering is defined in accordance with Kabat et al., *supra*. This numbering system is followed hereinafter. When the same data are analyzed by molecular modeling, the amino acid residues at these positions were shown to be in contact with the amino acid residues of CDR's in two thirds of the antibody variable regions that were examined.

These findings were used to define criterion (b) above. Specifically, if an amino acid position in an FR is predicted both to contact a CDR by molecular modeling and is frequently found experimentally to contact a CDR by X-ray crystallographic analysis, then the grafting of the amino acid residue of the donor is made a priority. In any other case, criterion (b) is not considered.

Similarly, with respect to criterion (d), X-ray crystallography data from the variable regions of a number of antibodies indicates that the amino acid residues at positions 36, 38, 43, 44, 46, 49, 87 and 98 in light chains and those at positions 37, 39, 45, 47, 91, 103 and 104 in heavy chains are frequently involved in the contact between heavy and light chains. If any of these amino acids are predicted to be involved in light and heavy chain contact by molecular modeling, then grafting of the amino acid residue of the donor is given priority. In any other case, criterion (d) is not considered.

In the construction of the humanized antibody based upon the acceptor 8E10'CL in Examples 9-15, both of the heavy chain

and the light chain of the antibody of the present invention are designed so that only CDR's from HFE7A as a donor may be grafted. Accordingly, the requirements a) to d) described above are not taken into consideration.

DNA encoding the variable regions of the H and L chains of a humanized anti-human Fas antibody of the present invention may be prepared in a number of ways.

In one method, polynucleotide fragments of between 60 and 70 nucleotides in length may be synthesized which represent partial nucleotide sequences of the desired DNA. The synthesis process is arranged such that the ends of fragments of the sense strand alternate with those of the antisense strand. The resulting polynucleotide fragments can be annealed to one another and ligated by DNA ligase. In this way the desired DNA fragment encoding the variable regions of the H and L chains of the humanized anti-human Fas antibody may be obtained.

Alternatively, DNA coding for the entire variable region of the acceptor may be isolated from human lymphocytes. Site directed mutagenesis, for example, may be used to introduce restriction sites into the regions encoding the CDR's of the donor. The CDR's may then be excised from the acceptor using the relevant restriction enzyme. DNA encoding the CDR's of the donor can then be synthesized and ligated into the acceptor molecule, using DNA ligase.

We prefer that DNA encoding the variable regions of the heavy and light chains of a desired humanized anti-human Fas antibody is obtained by the technique of overlap extension PCR [Horton, et al., (1989), Gene, 77, 61-68, incorporated herein by reference].

Overlap extension PCR allows two DNA fragments, each coding for a desired amino acid sequence, to be joined. For the sake of example, the two fragments are herein designated as (A) and (B). A sense primer (C) of 20 to 40 nucleotides which anneals with a 5'- region of (A) is synthesized, along with an antisense primer of 20 to 40 nucleotides (D), which anneals with a 3'- region of (B). Two further primers are required. First, a chimaeric sense primer (E), which comprises 20 to 30 nucleotides from a 3'- region of (A) joined to 20 to 30 nucleotides from a 5'- region of (B). Secondly, an antisense primer (F) is required, complementary to the sense primer.

A PCR reaction may be carried out using primers (C) and (F), in combination with a DNA template containing fragment A. This allows a DNA product to be produced comprising 20 to 30 nucleotides of the 5'- region of (B) joined to the 3'-end of (A). This fragment is termed fragment (G).

Similarly, PCR may be carried out using primers (D) and (E), in combination with a DNA template containing fragment B. This allows a DNA product to be produced comprising 20 to 30 nucleotides of the 3'- region of (A) joined to the 5'-end of (B). This fragment is termed fragment (H).

The (G) and (H) fragments carry complementary sequences of 40 to 60 nucleotides in the 3'- region of (G) and 40 to 60 nucleotides in the 5'-region of (H), respectively. A PCR reaction may be carried out using a mixture of the (G) and (H) fragments as a template. In the first denaturation step, the DNA becomes single stranded. Most of the DNA returns to the original form in the subsequent annealing step. However, a part of the DNA forms a heterologous DNA duplex, due to the annealing of (G) and (H) fragments in the region of sequence overlap. In the subsequent extension step, the protruding single-stranded

portions are repaired to result in chimaeric DNA which represents a ligation of (A) and (B). This DNA fragment is hereinafter referred to as (I). Fragment (I) can be amplified using primer (C) and primer (D).

In embodiments of the present invention, fragments (A) and (B) may represent DNA encoding the CDR regions of the H and L chains of a mouse humanized anti-human Fas monoclonal antibody, DNA coding for the FR regions of human IgG or DNA coding for the secretion signal of human IgG.

The codon or codons which correspond to a desired amino acid are known. When designing a DNA sequence from which to produce a protein, any suitable codon may be selected. For example, a codon can be selected based upon the codon usage of the host. Partial modification of a nucleotide sequence can be accomplished, for example by the standard technique of site directed mutagenesis, utilizing synthetic oligonucleotide primers encoding the desired modifications [Mark, D. F., et al., (1984), Proc. Natl. Acad. Sci. USA, 81, 5662-5666]. By using selected primers to introduce a specific point mutation or mutations, DNA coding for the variable regions of the H and L chains of any desired humanized anti-human Fas antibody can be obtained.

Integration of DNA of the present invention thus obtained into an expression vector allows transformation of prokaryotic or eukaryotic host cells. Such expression vectors will typically contain suitable promoters, replication sites and sequences involved in gene expression, allowing the DNA to be expressed in the host cell.

In general, three transformant strains carrying DNA encoding the variable regions of light chains of humanized anti-

**Fas antibodies**

, wherein human monoclonal antibody 8E10 is used as an acceptor, namely *E. coli* pHSGMM6 SANK 73697, *E. coli* pHSGHM17 SANK 73597, *E. coli* pHSGHH7 SANK 73497, as well as a transformant strain carrying DNA encoding the variable region of the heavy chain of the same humanized anti-Fas antibody, namely *E. coli* pgHSL7A62 SANK73397 were deposited in the Kogyo Gijutsuin Seimei-Kogaku Kogyo Gijutsu Kenkyujo on August 22, 1997, in accordance with the Budapest Treaty, and the accession numbers FERM BP-6071, FERM BP-6072, FERM BP-6073, and FERM BP-6074, respectively, were accorded them. Furthermore, two transformant strains carrying DNA encoding the light chains of the same humanized anti-Fas antibodies of the same *E. coli* pHSHM2 SANK 70198 and *E. coli* pHSHH5 SANK 70398, as well as a transformant strain carrying DNA encoding the heavy chain of the same humanized anti-Fas antibody, namely *E. coli* pgHPDHV3 SANK 70298 were deposited in the Kogyo Gijutsuin Seimei-Kogaku Kogyo Gijutsu Kenkyujo on February 26, 1998, in accordance in the Budapest Treaty, and the accession numbers FERM-6272, FERM-6274 and FERM BP-6273, respectively, were accorded. Therefore, DNA encoding each subunit of the humanized anti-Fas antibody protein can be obtained, for example, by isolating a plasmid from these deposited strains, or by performing PCR using an extract of the deposited strains as a template. The antibody of the present invention can be produced by expressing, in a host cell, DNA obtained by modifying the above mentioned DNA's by, for example, the overlap extension PCR described above.

Three transformant strains carrying DNA encoding the variable regions of the light chains of humanized anti-Fas antibodies of the present invention, namely *E. coli* pHSGLEU15-29-1 SANK 72598, *E. coli* pHSGLEU21-28-8 SANK 72698, *E. coli* pHSGLEU31-6-2 SANK 72798, as well as three transformant strains

carrying DNA encoding the variable region of the heavy chain of the same humanized anti-Fas antibody, namely *E. coli* pHSGAB580-3-21 SANK 72898, *E. coli* pHSGHEU222-1-2 SANK 73098, *E. coli* pHSGHEU223-30-1 SANK 72998, were deposited in the Kogyo Gijutsuin Seimei-Kogaku Kogyo Gijutsu Kenkyujo on September 18, 1998, in accordance with the Budapest Treaty, and the accession numbers FERM BP-6512, FERM BP-6511, FERM BP-6513, FERM BP-6515, FERM BP-6514 and FERM BP-6516, were accorded them respectively. Therefore, DNA encoding each subunit of the humanized anti-Fas antibody protein of the present invention can be obtained, for example, by isolating a plasmid from these deposited strains, or by performing PCR using an extract of the deposited strains as the template. A further transformant strain carrying DNA encoding heavy chains of humanized anti-Fas antibodies of the present invention, namely *E. coli* pgHSHHH1 SANK 72198, was deposited in the Kogyo Gijutsuin Seimei-Kogaku Kogyo Gijutsu Kenkyujo on September 18, 1998, in accordance with the Budapest Treaty, and the accession number FERM BP-6510 was accorded thereto.

A high purity, recombinant, anti-Fas antibody can be readily produced in high yields by the methodology described above.

In order to check that a recombinant anti-Fas antibody, prepared as above, specifically binds Fas, ELISA may be performed in a manner similar to that described above for the evaluation of antibody titers in immunized mice.

The HFE7A antibody, and humanized anti-Fas antibodies of the present invention, has the various functional properties a) to f) below, each of which may be verified by, for example, a method described.

Inducing apoptosis in T cells expressing Fas.

Apoptosis-inducing activity in T cells expressing Fas may be assayed by removing the thymus from a mouse which has been given a humanized anti-Fas antibody of the present invention (also referred to hereinbelow as "the antibody"), disrupting the thymus and contacting the cells obtained with T cells and an antibody specific for mouse Fas, and measuring the proportion of the cells to which both antibodies bind by flow cytometry.

Amelioration of the autoimmune symptoms of MRL gld/gld mice.

The antibody is intraperitoneally administered to a MRL gld/gld mouse. These mice carry a mutation in the gene coding for Fas ligand and exhibit symptoms resembling autoimmune diseases [c.f. Shin Yonehara (1994), Nikkei Science Bessatsu, 110, 66-77]. The antibody is capable, in many instances, of preventing, or at least ameliorating, swelling of the limbs, which is one of the autoimmune disease-like symptoms.

Failure to induce hepatic disorders.

Peripheral blood is drawn from a BALB/c mouse which has been given the antibody and blood levels of the enzymes glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) are measured, using an automated analyzer (for example, Model 7250; Hitachi Seisakusyo, K. K.) together with the reagent for the analyzer (for example, transaminase-HRII; Wako Pure Chemical Industries, Ltd.). Failure to cause elevated blood GOT and GPT levels indicate that the antibody does not induce hepatic disorders upon administration *in vivo*.

Therapeutic or prophylactic effect on fulminant hepatitis.

In an experimental system in which fulminant hepatitis is induced in mice by administering the anti-mouse Fas monoclonal antibody Jo2, the effects of administration of the above antibody simultaneously with Jo2 or after administration of Jo2

can be examined. Antibodies of the invention can prevent, to a large degree, all of the effects of Jo2 in mice, thereby demonstrating a protecting effect in the liver.

Preventative effect on the onset of collagen-induced arthritis. The effects of administration of the antibody on a rheumatoid arthritis model elicited by administering to a mouse an emulsion comprising collagen and Freund's complete adjuvant are examined. The antibody has prophylactic properties.

Induction of apoptosis in synovial cells from a rheumatoid arthritis patient.

Synovial cells obtained from an affected region of a patient with rheumatoid arthritis are cultured and the viability of the cells when the above antibody is contained in the culture medium is examined. Surprisingly, proliferation of the synovial cells is inhibited.

Thus, antibodies of the present invention, unlike previous, known, anti-Fas monoclonal antibodies, not only protect normal cells, but also kill abnormal cells. Accordingly, they are useful as prophylactic and therapeutic agents for diseases attributable to abnormalities of the Fas/Fas ligand system.

The ability of the proteins of the present invention to induce apoptosis can be established, for example, by culturing cells such as the human lymphocyte cell line HPB-ALL [Morikawa, S., et al, (1978), Int. J. Cancer, 21, 166-170] or Jurkat (American Type Culture No. TIB-1520) in medium in which the test sample has been or will be added. The survival rate may then be determined by, for example, an MTT assay [Green, L. M., et al., (1984), J. Immunological Methods, 70, 257-268].

Antibodies of the present invention can be used in various pharmaceutical preparations in respect of the various disease conditions connected with abnormalities of the Fas/Fas ligand system, such as those listed above.

Such a prophylactic or therapeutic agent may be administered in any of a variety of forms. Suitable modes of administration include oral administration, such as by tablets, capsules, granules, powders and syrups, or parenteral administration, such as by any suitable form of injection, including intravenous, intramuscular and intradermal, as well as infusions and suppositories. Thus, the present invention also provides methods and therapeutic compositions for treating the conditions referred to above. Such compositions typically comprise a therapeutically effective amount of the protein of the present invention in admixture with a pharmaceutically acceptable carrier therefor, and may be administered in any suitable manner, such as by parenteral, intravenous, subcutaneous or topical administration.

In particular, where the condition to be treated is local, then it is preferred to administer the protein as close as possible to the site. For example, serious rheumatic pain may be experienced in major joints, and the protein may be administered at such locations.

Systemically administered proteins of the present invention are particularly preferably administered in the form of a pyrogen-free, therapeutically, particularly parenterally, acceptable aqueous solution. The preparation of such pharmaceutically acceptable protein solutions with regard to aspects such as pH, isotonicity, stability and the like, is well within the skill of the person skilled in the art. In addition, the compositions of the present invention may comprise such

further ingredients as may be deemed appropriate, such as cell growth retardants and other medicaments.

It will be appreciated that the dosage will vary, depending on factors such as the condition, age and body weight of the patient, but usually the dosage for oral administration to an adult ranges between about 0.1 mg and 1,000 mg per day, which may be administered in a single dose or several divided doses. The dosage for parenteral administration typically ranges between 0.1 mg and 1,000 mg, which may be administered by a subcutaneous, intramuscular or intravenous injection (or injections).

A suitable oral administration form of the humanized anti-Fas antibody of the present invention is as an ampoule of a sterile solution or suspension in water or a pharmaceutically acceptable solution. Alternatively, a sterile powder (preferably, prepared by lyophilization of the humanized anti-Fas antibody) may be filled into an ampoule, which may then be diluted with a pharmaceutically acceptable solution for use.

Owing to the fact that the antibodies of the present invention used in human treatment have been humanized, toxicity is very low.

The present invention will now be illustrated by the following Examples. It will be understood that the scope of the present invention is not limited by these Examples.

Any methods, preparations, solutions and such like which are not specifically defined may be found in 'Molecular cloning - A laboratory Handbook' (*supra*, incorporated herein by reference). All solutions are aqueous and made up in sterile deionised water, unless otherwise specified.

REFERENCE EXAMPLE 1Preparation of Fas Antigen

In order to obtain a soluble version of human Fas lacking the transmembrane domain, an expression vector was constructed. This vector was designed to encode a fusion protein (the "Fas fusion protein") comprising the extracellular domain of human Fas fused to the extracellular domain of the mouse interleukin 3 (IL3) receptor [c.f. Gorman, D. M. et al., (1990), Proc. Natl. Acad. Sci. USA, 87, 5459-5463]. DNA encoding the human Fas fusion protein was prepared from this vector by PCR. The construction of the vector and preparation of DNA was as follows.

a) Template

The templates used for the PCR to construct the insert encoding the fusion protein were two plasmids. The first plasmid, pME18S-mFas-AIC [c.f. Nishimura, Y. et al., (1995), J. Immunol. 154, 4395-4403], was a DNA expression plasmid vector encoding a fusion protein, comprising the extracellular domain of mouse Fas and the extracellular domain of the mouse IL3 receptor. The second plasmid, pCEV4 [c.f. Itoh, N., et al., (1991), Cell, 66, 233-243], carried cDNA encoding human Fas.

b) PCR Primers

The following oligonucleotide primers were synthesized:

5'-GGGGAATTCC AGTACGGAGT TGGGGAAAGCT CTTT-3'

(N1: SEQ ID No.12 of the Sequence Listing);

5'-GTTTCTTCTG CCTCTGTCAC CAAGTTAGAT CTGGA-3'

(C3N: SEQ ID No.13 of the Sequence Listing);

5'-TCCAGATCTA ACTTGGTGAC AGAGGCAGAA GAAAC-3'

(N3N: SEQ ID No.14 of the Sequence Listing); and

5'-CCCTCTAGAC GCGTCACGTG GGCATCAC-3'

(CTN2: SEQ ID No.15 of the Sequence Listing).

Unless otherwise specified, all oligonucleotides in these Examples were synthesized using an automated DNA synthesizer (Model 380B; Perkin Elmer Japan, Applied Biosystems Division) following the instructions supplied with the manual [c.f. Matteucci, M. D. and Caruthers, M. H., (1981), J. Am. Chem. Soc., 103, 3185-3191]. After synthesis, each oligonucleotide (primer) was removed from the support, deprotected, and the resulting solution lyophilized to obtain a powder. This powder was then dissolved in distilled water and stored at -20°C until required.

c) First Stage of PCR

i) A DNA fragment, designated HFAS and encoding the extracellular domain of human Fas, was prepared as follows. PCR was performed using the LA (Long and Accurate) PCR Kit (Takara Shuzo Co., Ltd., Japan).

Composition of the PCR reaction solution:

template pCEV4 DNA, 20 ng;  
primer N1, 0.5 µg;  
primer C3N, 0.5 µg;  
10x concentrated LA PCR buffer (provided with the kit), 25 µl;  
dNTP's (provided with the kit), 25 µl; and  
LA Taq polymerase (provided with the kit), 12.5 units.

Sterile distilled water was added to the solution to a total volume of 250 µl. Unless otherwise specified, dNTP's are provided as an equimolar mixture of dATP, dCTP, dGTP and dTTP.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for

1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes (in all PCR reactions described in the Reference Examples, the temperature was regulated using the GeneAmp PCR system 9600; Perkin Elmer, Japan).

ii) A DNA fragment, designated MAIC and encoding the extracellular domain of the mouse IL3 receptor, was prepared as follows.

Composition of the PCR reaction solution:

template pME18S-mFas-AIC DNA, 20 ng;  
primer N3N, 0.5 µg;  
primer CTN2, 0.5 µg;  
10-fold concentrated LA PCR buffer, 25 µl;  
dNTP's, 25 µl;  
LA Taq polymerase, 12.5 units; and  
Sterile distilled water to a total volume of 250 µl.

The PCR reaction was conducted as above.

The amplified HFAS and MAIC DNA fragments, thus obtained, were separately first subjected to phenol extraction, then to ethanol precipitation [these two processes are defined in Example 2 (2) 3) a) below], after which the purified fragments were electrophoresed on a 5% w/v polyacrylamide gel. The gel was stained with 1 µg/ml of ethidium bromide to show up DNA under UV light. The bands determined to contain the desired DNA fragments were cut out using a razor blade and the DNA was electroeluted therefrom using an Amicon Centrifruter equipped with the centrifuge tube-type ultrafiltration device Centricon-10 (Amicon). After electroelution, the Centricon-10 unit containing the eluate was discarded and centrifuged at 7,500 x g for about 1 hour to concentrate

the DNA. The DNA was precipitated with ethanol and then dissolved in 20  $\mu$ l of distilled water.

d) Second stage of PCR

The FASAIC DNA fragment encoding the human Fas fusion protein (human Fas/murine IL3 receptor) was prepared as follows.

Composition of the PCR reaction solution:

template DNA solution HFAS, 20  $\mu$ l;  
template DNA solution MAIC, 20  $\mu$ l;  
primer N1, 0.5  $\mu$ g;  
primer CTN2, 0.5  $\mu$ g;  
10-fold concentrated LA PCR buffer, 25  $\mu$ l;  
dNTP's, 25  $\mu$ l;  
LA Taq polymerase, 12.5 units; and  
Sterile distilled water to a total volume of 250  $\mu$ l.

The PCR reaction was conducted as in c) above.

The amplified FASAIC DNA fragment, thus obtained, was first extracted with phenol, then precipitated with ethanol, after which it was electrophoresed on a 1% w/v polyacrylamide gel. The gel was stained with 1  $\mu$ g/ml of ethidium bromide to show up DNA under UV light. The band determined to contain the desired DNA fragment was cut out using a razor blade and the DNA was electroeluted therefrom using an Amicon Centrifiruter equipped with a Centricon-10 device, as described above. After electroelution, the Centricon-10 unit containing the eluate was removed and centrifuged at 7,500  $\times$  g for about 1 hour to concentrate the DNA, and the DNA was then precipitated with ethanol and finally dissolved in 50  $\mu$ l of distilled water.

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e) Construction of vectors

The whole of the FASAIC DNA, obtained in d) above, was digested with the restriction enzymes EcoRI and XbaI, then extracted with a phenol/chloroform mixture (50% v/v phenol saturated with water, 48% v/v chloroform, 2% v/v isoamyl alcohol), then precipitated with ethanol. The resulting precipitate was suspended in 2 µl of sterile deionized water.

Two micrograms of plasmid pME18S-mFas-AIC were digested with the restriction enzymes EcoRI and XbaI and dephosphorylated [the dephosphorylation process is as defined in Example 2 (2) 3) a) below]. The resulting DNA fragment was then ligated with the restriction-digested FASAIC DNA obtained above using a ligation kit (Takara Shuzo Co., Ltd.). The ligation product was then used in the transformation of *E. coli* strain DH5 $\alpha$  (Gibco BRL) as described by Hanahan [Hanahan, D., (1983), J. Mol. Biol., 166, 557-580]. Plasmid was then obtained from the transformed *E. coli* by the alkaline-SDS method [c.f. Maniatis, T., et al., (1989), in Molecular Cloning: A Laboratory Manual (2nd Edition), Cold Spring Harbor Laboratory, NY]. The plasmid thus obtained was designated phFas-AIC2.

This plasmid was next further purified using a large scale plasmid preparation kit (MaxiPrep DNA purification system, Promega). 20 µg of purified plasmid DNA was precipitated with ethanol and the precipitate was dissolved in 20 µl of sterile Dulbecco's PBS(-) medium (hereinafter referred to as PBS; Nissui Pharmaceutical Co., Ltd.).

f) Expression

COS-1 cells (American Type Culture Collection No. CRL-1650) were grown to semi-confluence in a culture flask (culture area: 225 cm<sup>2</sup>;

Sumitomo Bakelite, K. K.) containing Dulbecco's modified Eagle medium (DMEM; Nissui Pharmaceutical Co., Ltd., Japan) supplemented with 10% v/v fetal calf serum (FCS; Gibco) at 37°C under an atmosphere of 5% v/v gaseous CO<sub>2</sub>. The growth medium was then discarded, and 3 ml of an aqueous solution of 5 g/l trypsin and 2 g/l ethylenediaminetetraacetic acid (trypsin-EDTA solution; Sigma Chemicals, Co.) was added to the flask, which was then incubated at 37°C for 3 minutes to detach the cells from the flask.

The harvested cells were suspended in PBS, washed twice with PBS, and adjusted to 6 × 10<sup>7</sup> cells/ml with PBS. Twenty µl of the resulting cell suspension (1.2 × 10<sup>6</sup> cells) were mixed with 20 µl of the plasmid solution prepared above, and the mixture was introduced into a chamber with electrodes set 2 mm apart (Shimadzu Seisakusyo, K. K.). The chamber was next loaded into gene transfection apparatus (GTE-1; Shimadzu Seisakusyo, K. K.) and pulses of 600 V, duration 30 µsec, were applied twice, 1 second apart. The cell-DNA mixture in the chamber was then introduced into 10 ml of DMEM supplemented with 10% v/v FCS and incubated in a culture flask (culture area: 75 cm<sup>2</sup>) under 7.5% v/v CO<sub>2</sub> at 37°C for 24 hours. After this time, the culture supernatant was discarded and the cells were washed with serum-free DMEM. Subsequently, 10 ml of serum-free DMEM were added to the washed cells and the mixture was further incubated under 7.5% v/v CO<sub>2</sub> at 37°C for 24 hours, after which time the supernatant was recovered.

The recovered supernatant was dialyzed against 10 mM Tris-HCl (pH 8.0) in a dialysis tube (exclusion m.w. 12,000 - 14,000; Gibco BRL), and human Fas fusion protein was then further partially purified using FPLC apparatus by Pharmacia under the following conditions:

Column: Resource Q column (trademark; diameter (ϕ) 6.4 × 30 mm;

Pharmacia);

Eluent: 10 mM Tris-HCl (pH 8.0);

Flow rate: 5 ml/min;

Elution: NaCl 0.1 M - 0.3 M, linear gradient in 30 minutes.

The eluate was collected in fractions of 5 ml and these were assayed for Fas gene expression product by ELISA (Enzyme-Linked Immunosorbent Assay), as described below. First, 100 µl of each fraction were separately placed into wells of a 96-well microplate (Costar) and incubated at 37°C for 1 hour. After this time, the solution in the wells was tipped off, and the plate was washed 3 times with 100 µl/well of PBS containing 0.1% v/v Tween 20 (PBS-Tween). After washing, PBS containing 2% w/v bovine serum albumin ("BSA") was added in quantities of 100 µl/well, and the plate was then incubated at 37°C for 1 hour.

After this time, the wells were washed a further 3 times with 100 µl/well of PBS-Tween, after which 100 µl/well of a solution of anti-mouse IL-3 receptor β subunit monoclonal antibody HC (1 mg/ml; Igaku Seibutsugaku Kenkyujo, K. K.) diluted 100-fold with PBS-Tween was added to each well, and the plate was once again incubated at 37°C for 1 hour. The wells were then washed 3 times with 100 µl/well of PBS-Tween, and then 100 µl/well of horse radish peroxidase-labeled anti-mouse immunoglobulin antibody (Amersham) diluted 2000-fold with PBS-Tween was added to each well, and the plate was incubated at 37°C for another 1 hour, after which each well was again washed 3 times with 100 µl PBS-Tween. Horse radish peroxidase substrate (BioRad) was then added in a quantity of 100 µl/well and left for 5 minutes. After this time, the absorbance at 415 nm was measured with a microplate reader (Model 450; BioRad). The 19th to 23rd fractions, inclusive, which had high absorbance values at this wavelength, were collected to prepare the crude human Fas fusion protein sample.

REFERENCE EXAMPLE 2Immunization of mice and preparation of hybridoma(2-1) Immunization

A sample of 1 ml of the crude human Fas fusion protein solution obtained in Reference Example 1 above (total protein: 100 µg) was taken and, to this, were added 25 µl of 2N HCl, 250 µl of 9% w/v potash alum (final concentration: 1.1% w/v) and 25 µl of 2N NaOH. The resulting mixture was adjusted to a pH of between about 6.5 and 7.0 by the addition of about 120 µl of an aqueous solution of 10%(w/v) sodium hydrogencarbonate and left to stand at room temperature for about 30 minutes. After this time, 200 µl of killed *Bordetella pertussis* (Wako Pure Chemical Industries, Ltd.;  $1.2 \times 10^{11}$  cells/ml) were added to the mixture in order to activate the T cells, and the mixture was administered intraperitoneally to a Fas knock-out mouse. The mouse used was prepared in accordance with the method described by Senju et al. [c.f. Senju, S. et al., (1996), International Immunology, 8, 423]. The mouse was given an intraperitoneal booster injection, after 2 weeks, of crude human Fas fusion protein only (20 µg protein / mouse).

(2-2) Cell fusion

On the third day after the booster injection, the spleen was removed form the mouse and put into 10 ml of serum-free RPMI 1640 medium (10.4 g/l RPMI 1640 "Nussui" 1; Nissui Pharmaceutical Co., Ltd.) containing 20 mM HEPES buffer (pH 7.3), 350 mg/ml sodium hydrogencarbonate, 0.05 mM β-mercaptoethanol, 50 units/ml penicillin, 50 µg/ml streptomycin and 300 µg/ml L-glutamic acid, and disrupted by passing the organ through a mesh (Cell Strainer; Falcon) using a spatula. The resulting cell suspension was centrifuged to pelletize the spleen cells which were then washed twice with serum-free RPMI medium.

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The washed cells were then suspended in serum-free RPMI medium and counted.

In the meantime, myeloma NS1 cells (American Type Culture Collection TIB-18) had been grown to a cell density not exceeding  $1 \times 10^8$  cells/ml in ASF104 medium (Ajinomoto, K. K.) containing 10% v/v FCS (Gibco BRL) ("ASF medium with serum") at 37°C under 5% v/v CO<sub>2</sub>, and these were likewise disrupted, washed, suspended and counted.

An amount of the NS1 cell suspension calculated to contain  $3 \times 10^7$  cells was mixed with an amount of the spleen cell suspension calculated to contain  $3 \times 10^8$  cells. The resulting mix was centrifuged and the supernatant discarded. The following steps of cell fusion were performed whilst, all the time, keeping the plastic tube containing the pellet at 37°C in a beaker of warm water.

One ml of 50% (w/v) polyethylene glycol 1500 (Boehringer Manheim) was then slowly added to the tube, all the while stirring the pellet using the tip of a pipette. Subsequently, 1 ml of serum-free RPMI medium, prewarmed to 37°C, was slowly added in 2 portions, followed by the addition of a further 7 ml of serum-free RPMI medium. The resulting mix was then centrifuged, the supernatant was discarded and 10 ml of hypoxanthine aminopterin thymidine medium ("HAT medium"; Boehringer Manheim) containing 10% v/v FCS were added while stirring gently with the tip of a pipette. A further 20 ml of HAT medium containing 10% v/v FCS was added, and the suspension was dispensed into 96-well cell culture microplates at 100 µl/well and incubated at 37°C under 5% v/v CO<sub>2</sub>. After 7 or 8 days, 100 µl/well of fresh HAT medium were used to replace medium in any wells exhibiting a yellowish hue. The fusion cells from these wells were screened by limiting dilution as described below.

(2-3) Limiting dilution

Thymuses from 4 to 10 week old female BALB/c mice (from Japan SLC, Inc.) were removed, disrupted on a mesh (Cell Strainer; Falcon) as described above, and the disrupted cells were washed twice with hypoxanthine thymidine medium ("HT medium"; Boehringer Manheim) containing 10% v/v FCS. An amount of thymus cells corresponding to those from one mouse were suspended in 30 ml of HT medium containing 10% v/v FCS to produce a feeder cell suspension. The fusion cell preparation obtained above (2-2) was diluted with this feeder cell suspension 10- to 100-fold, and further diluted serially with feeder cell suspension to make suspensions having fusion cell densities of 5, 1 and 0.5 cells/ml. The thus prepared samples were dispensed into wells of 96-well cell culture microplates at 100 µl/well and incubated for 5 days at 37°C under 5% v/v CO<sub>2</sub>.

(2-4) Screening

WR19L12a cells [c.f. Itoh, N. et al., (1991), Cell, 66, 233-243] were propagated by incubation in RPMI 1640 medium containing 10% v/v FCS at 37°C under 5% v/v CO<sub>2</sub>. WR19L12a cells are derived from mouse T lymphoma WR19L cells (American Type Culture Collection TIB-52) and have been modified to express a gene encoding human Fas. The suspension of propagated WR19L12a cells was adjusted to a cell density of  $1 \times 10^7$  cells/ml and aliquots of 50 µl/well were dispensed into the wells of a 96-well microplate, the wells having U-shaped bottoms (Nunc) and the plate was centrifuged (90 x g, 4°C, 10 minutes). The supernatant was discarded and 50 µl/well of culture supernatant obtained from the fusion cells cultured in 2-3 above were added to the wells, with mixing.

The resulting mixtures were kept standing on ice for 1 hour and then centrifuged (90 x g, 4°C, 10 minutes), and the supernatant removed.

The pellets were each washed twice with 100 µl/well of flow cytometry buffer [PBS containing 5% v/v FCS and 0.04% (w/v) sodium azide]. A secondary antibody [50 µl of fluorescein-5-isothiocyanate (FITC) labeled goat anti-mouse IgG antibody IgG fraction (Organon Technika) diluted 500-fold] was added to the washed cells, and the mixture was kept standing on ice for 1 hour. After further centrifugation (90 x g, 4°C, 10 minutes), and removal of the supernatant, the pellet was washed twice with 100 µl/well of flow cytometry buffer, and the cells were fixed by adding 50 µl of 3.7% v/v formaldehyde solution and standing on ice for 10 minutes. After centrifugation (90 x g, 4°C, 10 minutes) and removal of the supernatant, the pellets were again washed with 100 µl/well of flow cytometry buffer, and suspended in a further 100 µl/well of flow cytometry buffer to produce the flow cytometry samples.

The intensity of FITC fluorescence of the cells in each sample was measured with a flow cytometer (Epics Elite; Coulter; excitation wave length: 488 nm; detection wave length: 530 nm) and fusion cells were selected from samples which had FITC fluorescence intensities clearly higher (FITC fluorescence intensities of about 100 to 1,000) than those for control WR19L12a cells to which no fusion cell supernatant had been added (FITC fluorescence intensity of about 0.3).

#### (2-5) Cloning

The steps described in (2-3) and (2-4) above were repeated 5 times for the cells selected in (2-4), thereby enabling the selection of several hybridoma clones which each produced a single antibody binding WR19L12a but not binding WR19L. Binding of these antibodies to mouse Fas was examined by using an assay similar to the one described in (2-4), but using L5178YAl cells. The L5178YAl cell line expresses murine Fas. L5178YAl is a cell line produced by transfecting L5178Y cells with

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a mouse Fas expression vector. L5178Y cells (American Type Culture Collection No. CRL-1722) express almost no Fas.

As a result of this selection procedure, a mouse-mouse hybridoma, designated HFE7A and producing an antibody binding to L5178YA1 cells, but not L5178Y cells, was obtained. This hybridoma, HFE7A, was deposited with Kogyo Gijutsuin Seimeい Kogaku Kogyo Gijutsu Kenkyujo on February 20, 1997, in accordance with the Budapest Treaty for the Deposit of Microorganisms, and has been assigned accession No. FERM BP-5828.

The subclass of the antibody produced by the mouse-mouse hybridoma HFE7A (hereinafter referred to simply as "HFE7A") was demonstrated to be IgG1, κ, after testing with a monoclonal antibody isotyping kit (Pierce).

REFERENCE EXAMPLE 3

Purification of HFE7A Monoclonal Antibody

The mouse-mouse hybridoma HFE7A obtained in Reference Example 2 (FERM BP-5828) was grown to a cell density of  $1 \times 10^6$  cells/ml by incubation in 1 l of ASF medium, containing 10% v/v FCS, at 37°C under 5% v/v CO<sub>2</sub>. The culture was then centrifuged (1,000 r.p.m., 2 minutes) and the supernatant discarded. The cell pellet was washed once with serum-free ASF medium, suspended in 1 l of serum-free ASF medium and incubated for 48 hours at 37°C under 5% v/v CO<sub>2</sub>. After this time, the culture was centrifuged (1,000 r.p.m. for 2 minutes) to recover the supernatant. This supernatant was then placed in a dialysis tube (exclusion m.w.: 12,000 - 14,000; Gibco BRL), and dialyzed against 10 volumes of 10 mM sodium phosphate buffer (pH 8.0). Partial purification of IgG from the inner solution was achieved using a high performance

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liquid chromatography apparatus (FPLC system; Pharmacia) under the following conditions:

column: DEAE-Sepharose CL-6B column (column size 10 ml;  
Pharmacia);  
eluent: 10 mM sodium phosphate buffer (pH 8.0);  
flow rate: 1 ml/min;  
elution: linear gradient of 1 M NaCl (0 to 50%, 180 min).

The eluate was collected in fractions of 5 ml and each fraction was assayed for anti-Fas antibody titer by ELISA using the human Fas fusion protein prepared above.

First, 100 µl/well of the crude human Fas fusion protein solution prepared in Reference Example 1 was introduced into the wells of a 96-well ELISA microplate. After incubation at 37°C for 1 hour, the solution was discarded and the wells were each washed 3 times with 100 µl/well of PBS-Tween. Then, 100 µl/well of PBS containing 2% BSA was added and incubated at 37°C for 1 hour. After this time, the cells were washed 3 times with 100 µl/well of PBS-Tween, and then 100 µl samples of the fractions to be assayed were added to the wells, and the plate incubated at 37°C for 1 hour. Next, after washing each of the wells 3 times with 100 µl/well of PBS-Tween, 100 µl/well of horse radish peroxidase labeled anti-mouse immunoglobulin antibody (Amersham), diluted 2000-fold with PBS-Tween, were added and allowed to react at 37°C for 1 hour. After this time, each well was washed 3 times with 100 µl/well PBS-Tween. Horse radish peroxidase substrate (BioRad) was added in a quantity of 100 µl/well and left for 5 minutes before reading the absorbance of each well at 415 nm with a microplate reader.

The 21st to 30th fractions, inclusive, which had high absorbance values, were pooled and applied to two antibody affinity purification columns (HighTrap Protein G column, column volume 5 ml; Pharmacia).

After washing the columns with equilibrium buffer [20 mM sodium phosphate buffer (pH 7.0), 25 ml/column], antibody was eluted with 15 ml per column of elution buffer [0.1 M glycine-HCl (pH 2.7)]. The eluate was collected in tubes each containing 1.125 ml of 1 M Tris-HCl (pH 9.0) and centrifuged at 3,000 × g at 4°C for 2 hours in the top of a centrifuge tube-type ultrafiltration device (CentriPrep 10; Grace Japan, K. K.) immediately after completion of elution. The filtrate recovered in the bottom of the device was discarded, and 15 ml of PBS was added to the top and the preparation was once again centrifuged at 3,000 × g at 4°C for 2 hours. These same steps were repeated five times, in all. The 5th centrifugation was stopped when the volume of the solution remaining in the top reached 0.5 ml, and this was retained as the HFE7A sample.

Reference Example 4

cDNA Cloning

(4-1) Preparation of poly(A)<sup>+</sup> RNA

Cells of the mouse-mouse hybridoma HFE7A (FERM BP-5828), obtained in Reference Example 2, were grown to a cell density of  $1 \times 10^6$  cells/ml in 1 l of ASF medium supplemented with 10% v/v FCS at 37°C under 5% v/v CO<sub>2</sub>. These cells were harvested by centrifugation and lysed in the presence of guanidinium thiocyanate solution [4 M guanidinium thiocyanate, 1% v/v Sarcosyl, 20 mM EDTA, 25 mM sodium citrate (pH 7.0), 100 mM 2-mercaptoethanol, 0.1% v/v Antifoam A] and the lysate was recovered. Isolation of poly(A)<sup>+</sup> RNA was performed essentially as described in "Molecular Cloning A Laboratory Manual" [c.f. Maniatis, T., et al., (1982), pp. 196-198]. More specifically, the procedure was as follows.

The recovered cell lysate was sucked into and exhausted from a 10 ml-syringe equipped with a 21-gauge needle, several times. The cell lysate was layered over 3 ml of an aqueous solution of 5.7 M cesium chloride, 0.1 M EDTA solution (pH 7.5) in a polyallomer centrifuge tube for the bucket of a RPS-40T rotor (Hitachi Seisakusyo, K. K.). The lysate was then centrifuged at 30,000 r.p.m. at 20°C for 18 hours, and the resulting pellet was dissolved in 400 µl of distilled water and subjected to ethanol precipitation. The resulting precipitate was again dissolved in 400 µl of distilled water, mixed with an equal volume of a mixture of chloroform and 1-butanol (4:1, v/v), whereafter the aqueous layer was recovered after centrifugation at 5000 r.p.m. for 10 minutes. This aqueous layer was again precipitated with ethanol and the precipitate was dissolved in 600 µl of distilled water. The resulting solution was retained as the total RNA sample.

Poly (A)<sup>+</sup> RNA was purified from 600 µg (dry weight) of the total RNA sample, obtained above, by oligo(dT) cellulose chromatography.

More specifically, the total RNA was dissolved in 200 µl of adsorption buffer [0.5 M NaCl, 20 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.1% v/v sodium dodecyl sulfate (SDS)], then heated at 65°C for 5 minutes, and then applied to a column of oligo(dT) cellulose (Type 7; Pharmacia) which had been loaded with adsorption buffer. Poly(A)<sup>+</sup> RNA was eluted and recovered from the column using elution buffer [10 mM Tris-HCl (pH 7.5), 1 mM EDTA, 0.05% v/v SDS]. A total of 100 µg of poly(A)<sup>+</sup> RNA fraction was obtained by this procedure.

(4-2) Determination of the N-terminal amino acid sequences of the heavy and light chains of HFE7A

Ten microliters of the solution containing the anti-human Fas antibody HFE7A, obtained in Reference Example 3, was subjected to SDS-

polyacrylamide gel electrophoresis ("SDS-PAGE"), using a gel concentration of 12% w/v, 100 V constant voltage, for 120 minutes. After electrophoresis the gel was immersed in transfer buffer [25 mM Tris-HCl (pH 9.5), 20% methanol, 0.02% v/v SDS] for 5 minutes. After this time, the protein content of the gel was transferred to a polyvinylidene difluoride membrane ("PVDF membrane"; pore size 0.45 µm; Millipore, Japan), presoaked in transfer buffer, using a blotting apparatus (KS-8451; Marysol) under conditions of 10 V constant voltage, 4°C, for 14 hours.

After this time, the PVDF membrane was washed with washing buffer [25 mM NaCl, 10 mM sodium borate buffer (pH 8.0)], then stained in a staining solution (50% v/v methanol, 20% v/v acetic acid and 0.05% w/v Coomassie Brilliant Blue) for 5 minutes to locate the protein bands. The PVDF membrane was then destained with 90% v/v aqueous methanol, and the bands corresponding to the heavy chain (the band with the lower mobility) and light chain (the band with the higher mobility) previously located on the PVDF membrane were excized and washed with deionized water.

The N-terminal amino acid sequences of the heavy and light chains could now be determined by the Edman automated method [c.f. Edman, P., et al., (1967), Eur. J. Biochem., 1, 80] using a gas-phase protein sequencer (PPSQ-10; Shimadzu Seisakusyo, K. K.).

The N-terminal amino acid sequence of the band corresponding to the heavy chain was determined to be:

Gln-Xaa-Gln-Leu-Gln-Gln-Pro-Gly-Ala-Glu-Leu (SEQ ID No. 16 of the Sequence Listing);

and the N-terminal amino acid sequence of the band corresponding to the light chain was determined to be:

Asp-Ile-Val-Leu-Thr-Gln-Ser-Pro-Ala-Ser-Leu-Ala-Val-Ser-Leu-Gly-Gln-Arg-Ala-Thr-Ile-Ser (SEQ ID No. 17 of the Sequence Listing).

Comparison of these amino acid sequences with the database of amino acid sequences of antibodies produced by Kabat et al. [c.f. Kabat E. A., et al., (1991), in "Sequences of Proteins of Immunological Interest Vol.II," U.S. Department of Health and Human Services] revealed that the heavy chain ( $\gamma$ 1 chain) and the light chain ( $\kappa$  chain) of HFE7A belonged to subtypes 2b and 3, respectively. Based on these findings, oligonucleotide primers were synthesized which would be expected to hybridize with portions of the 5'-untranslated regions and the very ends of the 3'-translated regions of the genes belonging to these mouse subtypes [c.f. Kabat et al., *ibid.*; Matti Kartinen et al., (1988), 25, 859-865; and Heinrich, G., et al., (1984), *J. Exp. Med.*, 159, 417-435]: 5'-GACCTCACCA TGGGATGGA-3' (H1: SEQ ID No. 18 of the Sequence Listing); 5'-TTTACCAAGGA GAGTGGGAGA-3' (H2: SEQ ID No. 19 of the Sequence Listing); 5'-AAGAACGCATC CTCTCATCTA-3' (L1: SEQ ID No. 20 of the Sequence Listing); and 5'-ACACTCATTC CTGTTGAAGC-3' (L2: SEQ ID No. 21 of the Sequence Listing).

(4-3) cDNA Cloning

cDNA encoding the heavy and light chains of the mouse anti-human Fas monoclonal antibody HFE7A was cloned by a combination of reverse transcription and PCR ("RT-PCR"). Amplification was performed on the poly(A)<sup>+</sup> RNA fraction obtained from HFE7A-producing hybridoma cells as described in (4-1) above. The RT-PCR reaction was performed using RNA PCR Kit (AMV) Version 2 (Takara Shuzo Co., Ltd.).

a) The reverse transcriptase reaction

The oligonucleotide primer sets (5'-terminal and 3'-terminal primers), synthesized in (4-2) above, were used as primer pairs for the RT-PCR reaction for the heavy and light chains.

Composition of the reaction solution:

poly(A)<sup>+</sup> RNA (heavy or light chain, as required), 1 µg;  
3'-primer (H2 or L2), 0.3 µg;  
Tris-HCl (pH 8.3), 10 mM;  
potassium chloride, 50 mM;  
dNTP's, 1 mM;  
magnesium chloride, 5 mM;  
RNase inhibitor (provided with the kit), 0.5 unit;  
reverse transcriptase (provided with the kit), 0.25 unit; and  
redistilled water to a total volume of 20 µl.

The reaction solution was incubated at 55°C for 30 minutes, 99°C for 5 minutes and then 5°C for 5 minutes. The thus treated RT solution was then used in the following PCR stage.

b) PCR

Composition of the PCR reaction solution:

reverse transcriptase reaction solution, 20 µl;  
10-fold concentrated RNA PCR buffer (provided with the kit),  
10 µl;  
magnesium chloride solution (provided with the kit), 10 µl;  
Taq polymerase (provided with the kit), 2.5 units;  
5'-primer (H1 or L1), final concentration 0.2 µM; and  
sterile deionized water to a total volume of 100 µl.

The PCR reaction solution was heated at 94°C for 2 minutes, then followed by a cycle of 94°C for 30 seconds, 60°C for 30 seconds and 72°C for 1.5 minutes, repeated 28 times.

After the PCR reaction, aliquots of the reaction solutions were electrophoresed on 1.5% w/v agarose gels. Bands of about 1.4 kbp and about 0.7 kbp were found to have been amplified in the reaction solutions, using the primers for the heavy chain and those for the light chain, respectively. This confirmed that cDNA's encoding heavy and light chains had been amplified, as intended. Accordingly, the amplified PCR reaction solutions could be used in the next step of cloning the amplified cDNA's using the TA Cloning kit (Invitrogen). This was performed as follows.

The relevant PCR reaction solution, together with 50 ng of pCRII vector (provided with the TA Cloning kit), was mixed in 1 µl of 10× ligase reaction buffer [6 mM Tris-HCl (pH 7.5), 6 mM magnesium chloride, 5 mM sodium chloride, 7 mM β-mercaptoethanol, 0.1 mM ATP, 2 mM DTT, 1 mM spermidine, and 0.1 mg/ml bovine serum albumin], to which 4 units of T4 DNA ligase (1 µl) had been added. The total volume of the mixture was adjusted to 10 µl with sterile deionized water, and the resulting ligase solution was incubated at 14°C for 15 hours.

After this time, 2 µl of the ligase reaction solution was added to 50 µl of competent *E. coli* strain TOP10F' (provided with the TA Cloning kit and brought to competence in accordance with the kit's instruction manual) to which 2 µl of 0.5 M β-mercaptoethanol had been added, and the resulting mixture was kept on ice for 30 minutes, then at 42°C for 30 seconds, and again on ice for 5 minutes. Next, 500 µl of SOC medium (2% v/v tryptone, 0.5% w/v yeast extract, 0.05% w/v sodium chloride, 2.5 mM potassium chloride, 1 mM magnesium chloride, and 20 mM

glucose) was added to the culture, and the mixture was incubated for 1 hour at 37°C with shaking.

After this time, the culture was spread on an L-broth agar plate [1% v/v tryptone, 0.5% w/v yeast extract, 0.5% w/v sodium chloride, 0.1% w/v glucose, and 0.6% w/v bacto-agar (Difco)], containing 100 µg/ml ampicillin, and incubated at 37°C, overnight. Single ampicillin resistant colonies appearing on the plate were selected and scraped off with a platinum transfer loop, and cultured in L-broth medium containing 100 µg/ml ampicillin at 37°C, overnight, with shaking at 200 r.p.m. After incubation, the cells were harvested by centrifugation, from which plasmid DNA was prepared by the alkali method. The thus obtained plasmids were designated as plasmid pCR-H (the plasmid carrying cDNA encoding the heavy chain of HFE7A) or pCR-L (the plasmid carrying cDNA encoding the light chain of HFE7A).

#### (4-4) Nucleotide sequence analysis

The nucleotide sequences of both of the cDNA's encoding the heavy chain of HFE7A (1.4 kbp) and the light chain of HFE7A (0.7 kbp) carried by the plasmids pCR-H and pCR-L, obtained in (4-3) above, were determined by the dideoxy method [c.f. Sanger, F. S., et al., (1977), Proc. Natl. Acad. Sci. USA, 74:5463-5467] using a gene sequence analyzer (Model 310 Genetic Analyzer; Perkin Elmer, Japan).

The cDNA nucleotide sequences of the heavy and light chains of HFE7A, thus determined, are given as SEQ ID Nos. 8 and 10, respectively, in the Sequence Listing. The concomitant, complete amino acid sequences of the heavy and light chains of HFE7A, as coded by the cDNA's, are given as SEQ ID Nos. 9 and 11, respectively, of the Sequence Listing. The N-terminal amino acid sequence of HFE7A heavy chain established in (4-1) above (SEQ ID No. 16 of the Sequence Listing) matched perfectly

with the sequence of amino acid Nos. 1 to 11 of SEQ ID No. 9, except for the one uncertain residue. The N-terminal amino acid sequence of the HFE7A light chain (SEQ ID No. 17 of the Sequence Listing) matched exactly the sequence of amino acid Nos. 1 to 22 of SEQ ID No. 11. Thus, the N-termini of the mature heavy and light chain proteins of HFE7A were demonstrated to be amino acids Nos. 1 to 11 and Nos. 1 to 22 in SEQ ID Nos. 9 and 11, respectively.

Furthermore, when the amino acid sequences of the heavy and light chains were compared with the database of amino acid sequences of antibodies [Kabat E. A., et al., (1991), in "Sequences of Proteins of Immunological Interest Vol.II," U.S. Department of Health and Human Services], it was established that, for the heavy chain, amino acid Nos. 1 to 121 of SEQ ID NO. 9 constituted the variable region, while amino acid Nos. 122 to 445 constituted the constant region. For the light chain, amino acid Nos. 1 to 111 of SEQ ID NO. 11 constituted the variable region, while amino acid Nos. 112 to 218 constituted the constant region.

The locations and sequences of the CDR's in the amino acid sequences of the variable regions of the heavy and light chains of HFE7A, as determined above, were also elucidated by comparing the homologies with the same database of amino acid sequences of antibodies [*c.f.* Kabat E. A., et al., (1991), *ibid.*]. From this publication, it can be established the lengths of the framework regions in the variable regions are substantially the same, and that the amino acid sequences share common characteristics, among different antibodies of the same subtype. CDR's are unique sequences located between the framework regions. Therefore, by comparing the amino acid sequences of the heavy and light chains of HFE7A with those of the same subtypes in Kabat's work, it was possible to identify the CDR's of HFE7A.

Accordingly, it was established that, in the heavy chain of HFE7A (SEQ ID No. 9 in the Sequence Listing), amino acid Nos. 31 to 35 form CDRH<sub>1</sub>, amino acid Nos. 50 to 66 form CDRH<sub>2</sub> and amino acid Nos. 99 to 110 form CDRH<sub>3</sub>. The CDR's in the light chain of HFE7A (SEQ ID No. 11 in the Sequence Listing) were identified as amino acid Nos. 24 to 38 (CDRL<sub>1</sub>), amino acid Nos. 54 to 60 (CDRL<sub>2</sub>), and amino acid Nos. 93 to 101 (CDRL<sub>3</sub>).

REFERENCE EXAMPLE 5

Preparation of recombinant antibody

(5-1) Construction of expression plasmid

Recombinant expression vectors for animal cells were constructed by inserting the cDNA's encoding the heavy and light chains of HFE7A (cloned in Reference Example 4) into the expression vector pMS18S [c.f. Hara, T., et al., (1992), EMBO J., 11, 1875]. This was performed as follows.

First, oligonucleotide primers:

5'-GGGAATTCTG ACCTCACCAT GGGATGGA-3' (H3: SEQ ID No. 22 of the Sequence Listing) and

5'-GGGTCTAGAC TATTACCAAG GAGAGTGGGA GA-3' (H4: SEQ ID No. 23 of the Sequence Listing)

were synthesized. These primers serve for the introduction of a recognition site for the restriction enzyme EcoRI, for a recognition site for the restriction enzyme XbaI, as well as a termination codon, at the 5'-end and at the 3'-end, respectively, of the heavy chain cDNA carried by plasmid pCR-H.

Oligonucleotide primers:

5'-GGGGAATTCA AGAACATCC TCTCATCTA-3' (L3: SEQ ID No. 24 of the Sequence Listing) and

5'-GGGGCGGCCG CTTACTAACCA CTCATTCTG TTGAAGC-3' (L4: SEQ ID No. 25 of the Sequence Listing)

were also synthesized. These primers serve for the introduction of a recognition site for the restriction enzyme EcoRI, for a recognition site for the restriction enzyme NotI, as well as for a termination codon, at the 5'-end and at the 3'-end, respectively, of the light chain cDNA carried by plasmid pCR-L.

Using these respective primers for the heavy and light chains, PCR was performed as follows.

Composition of the reaction solution:

template (pCR-H or pCR-L), 1 µg;  
5'-primer (H3 or L3), 40 pmol;  
3'-primer (H4 or L4), 40 pmol;  
Tris-HCl (pH 8.0), 20 mM;  
potassium chloride, 10 mM;  
ammonium sulfate, 6 mM;  
magnesium chloride, 2 mM;  
Triton X-100, 0.1%;  
bovine serum albumin, nuclease-free, 10 µg/ml;  
dNTP's, 0.25 mM;  
native Pfu DNA polymerase (Stratagene), 5 units; and  
sterile distilled water to a total volume of 100 µl.

PCR thermal conditions:

Initial heating of the reaction solution was at 94°C for 2 minutes, after which a thermal cycle of 94°C for 30 seconds, 60°C for 30 seconds and 75°C for 1.5 minutes was repeated 28 times.

The resulting amplified DNA was digested with the restriction enzymes EcoRI and XbaI (for the heavy chain) or EcoRI and NotI (for the light chain), and then mixed with the animal cell expression plasmid pME18S [c.f. Hara. T., et al., (1992), EMBO J., 11, 1875] which had either been digested with the restriction enzymes EcoRI and XbaI (for the heavy chain) or EcoRI and NotI (for the light chain) and dephosphorylated using CIP [as described in Example 2 (2) 3) c) below]. One microliter of 4 units of T4 DNA ligase were added to 8 µl of the resulting mixture, and 1 µl of 10× ligase reaction buffer [6 mM Tris-HCl (pH 7.5), 6 mM magnesium chloride, 5 mM sodium chloride, 7 mM β-mercaptoethanol, 0.1 mM ATP, 2 mM DTT, 1 mM spermidine, and 0.1 mg/ml bovine serum albumin] was then also added to the mixture, which was then incubated at 14°C for 15 hours.

After this time, 2 µl of the incubated ligase reaction solution was mixed with 50 µl of competent *E. coli* strain JM109 at a cell density of 1-2 × 10<sup>9</sup> cells/ml (Takara Shuzo Co., Ltd.), and the mixture was kept on ice for 30 minutes, then at 42°C for 30 seconds, and again on ice for 5 minutes. Then, 500 µl of SOC medium (2% v/v tryptone, 0.5% w/v yeast extract, 0.05% w/v sodium chloride, 2.5 mM w/v potassium chloride, 1 mM magnesium chloride, and 20 mM glucose) was added to the mixture, which was incubated for a further hour, with shaking. Transformant strains were then isolated, and plasmid DNA was prepared from the strains, following the methods described in Reference Example 4 (4-3).

The resulting plasmids were designated pME-H (the expression plasmid vector carrying cDNA encoding the heavy chain of HFE7A) and pME-L (the expression plasmid vector carrying cDNA encoding the light chain of HFE7A). The transformant *E. coli* strains harboring these plasmids, designated as *E. coli* pME-H and *E. coli* pME-L, were deposited with Kogyo Gijutsuin Seimei-kogaku Gijutsu Kenkyujo on March 12, 1997, in accordance with the Budapest Treaty for the Deposit of Microorganisms,

and were accorded the accession numbers FERM BP-5868 and FERM BP-5867, respectively.

(5-2) Expression in COS-7 cells

Transfection of COS-7 cells with the expression plasmids pME-H and pME-L obtained in (5-1) above was performed by electroporation using a gene transfection apparatus (ECM600; BTX).

COS-7 cells (American Type Culture Collection No. CRL-1651) were grown up to semi-confluence in a culture flask (culture area: 225 cm<sup>2</sup>; Sumitomo Bakelite, K. K.) containing DMEM supplemented with 10% v/v FCS. Subsequently, the medium was discarded and 3 ml of trypsin-EDTA solution (Sigma Chemicals Co.) were added to the cells, followed by incubation at 37°C for 3 minutes. The cells detached by this process were harvested, washed twice with PBS and then adjusted to a cell density of 5 × 10<sup>6</sup> cells/ml with PBS.

Meanwhile, 20 µg each of plasmids pME-H and pME-L, prepared using a large-scale plasmid preparation kit (MaxiPrep DNA Purification System; Promega), were separately precipitated with ethanol and dissolved in 20 µl each of sterile PBS. Where COS-7 cells were cotransfected with both plasmids, 20 µg of each of the plasmids were used and dissolved together in 20 µl of sterile PBS.

Twenty µl of the cell suspension prepared above (1.2 × 10<sup>6</sup> cells) and 20 µl of the relevant plasmid solution were mixed and transferred to a chamber with electrodes set at a distance apart of 2 mm (BTX). The chamber was then loaded in the gene transfection apparatus and given a single pulse of 10 msec at 150 V to provide a total charge of 900 µF. The cell-DNA mixture in the chamber was added to 40 ml of DMEM supplemented with 10% v/v FCS and incubated in plastic cell culture

dishes under 5% v/v CO<sub>2</sub> at 37°C for 24 hours. After this time, the culture supernatant was discarded and the cells were washed with serum-free DMEM medium. After that, 40 ml of serum-free DMEM was added to each of the plastic dishes and the supernatant recovered after the cells had been cultured under 5% v/v CO<sub>2</sub> at 37°C for a further 72 hours.

Using the above method, COS-7 cells were obtained which were transfected with either or both plasmids (as shown below), and the supernatant of each of the transformants was recovered:

- (A) : pME-H only;
- (B) : pME-L only; and
- (C) : cotransfection of pME-H and pME-L.

(5-3) Detection of anti-Fas antibody in transformant culture supernatant

Expression of anti-Fas antibody in the culture supernatants obtained in (5-2) above was determined by ELISA, in a manner similar to that described in Reference Example 3, and using the human Fas fusion protein as the antigen. It was established that the production of an antibody reacting with the human Fas antigen fusion protein in the culture supernatant only happened when pME-H and pME-L were both used to cotransfect COS-7 cells [5-2 (C)].

REFERENCE EXAMPLE 6

Epitope Determination

(6-1) ELISA

The following peptides were synthesized by Fmoc solid phase synthesis [c.f. Carpino, L. A. and Han, G. Y., (1970), J. Am. Chem.

Soc., 92, 5748-5749] using an automated peptide synthesizer (Model 430A; Perkin Elmer, Japan, Applied Biosystems Division):

Arg-Leu-Ser-Ser-Lys-Ser-Val-Asn-Ala-Gln-Val-Thr-Asp-Ile-Asn-Ser-Lys-Gly-Leu (P1: SEQ ID No. 26 of the Sequence Listing);  
Val-Thr-Asp-Ile-Asn-Ser-Lys-Gly-Leu-Glu-Leu-Arg-Lys-Thr-Val-Thr-Val-Glu (P2: SEQ ID No. 27 of the Sequence Listing);  
Glu-Leu-Arg-Lys-Thr-Val-Thr-Val-Glu-Thr-Gln-Asn-Leu-Glu-Gly-Leu-His-His-Asp (P3: SEQ ID No. 28 of the Sequence Listing);  
Thr-Gln-Asn-Leu-Glu-Gly-Leu-His-His-Asp-Gly-Gln-Phe-Cys-His-Lys-Pro-Cys-Pro-Pro (P4: SEQ ID No. 29 of the Sequence Listing);  
Gly-Gln-Phe-Cys-His-Lys-Pro-Cys-Pro-Pro-Gly-Glu-Arg-Lys-Ala-Arg-Asp-Cys-Thr-Val (P5: SEQ ID No. 30 of the Sequence Listing);  
Gly-Glu-Arg-Lys-Ala-Arg-Asp-Cys-Thr-Val-Asn-Gly-Asp-Glu-Pro-Asp-Cys-Val-Pro-Cys-Gln (P6: SEQ ID No. 31 of the Sequence Listing);  
Asn-Gly-Asp-Glu-Pro-Asp-Cys-Val-Pro-Cys-Gln-Glu-Gly-Lys-Glu-Tyr-Thr-Asp-Lys-Ala (P7: SEQ ID No. 32 of the Sequence Listing);  
Glu-Gly-Lys-Glu-Tyr-Thr-Asp-Lys-Ala-His-Phe-Ser-Ser-Lys-Cys-Arg-Arg-Cys-Arg (P8: SEQ ID No. 33 of the Sequence Listing);  
His-Phe-Ser-Ser-Lys-Cys-Arg-Arg-Cys-Arg-Leu-Cys-Asp-Glu-Gly-His-Gly-Leu-Glu-Val (P9: SEQ ID No. 34 of the Sequence Listing);  
Leu-Cys-Asp-Glu-Gly-His-Gly-Leu-Glu-Val-Glu-Ile-Asn-Cys-Thr-Arg-Thr-Gln-Asn-Thr (P10: SEQ ID No. 35 of the Sequence Listing);  
Glu-Ile-Asn-Cys-Thr-Arg-Thr-Gln-Asn-Thr-Lys-Cys-Arg-Cys-Lys-Pro-Asn-Phe-Phe-Cys (P11: SEQ ID No. 36 of the Sequence Listing);  
Lys-Cys-Arg-Cys-Lys-Pro-Asn-Phe-Phe-Cys-Asn-Ser-Thr-Val-Cys-Glu-His-Cys-Asp-Pro (P12: SEQ ID No. 37 of the Sequence Listing);  
Asn-Ser-Thr-Val-Cys-Glu-His-Cys-Asp-Pro-Cys-Thr-Lys-Cys-Glu-His-Gly-Ile-Ile-Lys (P13: SEQ ID No. 38 of the Sequence Listing);  
Cys-Thr-Lys-Cys-Glu-His-Gly-Ile-Ile-Lys-Glu-Cys-Thr-Leu-Thr-Ser-Asn-Thr-Lys-Cys (P14: SEQ ID No. 39 of the Sequence Listing);  
Glu-Cys-Thr-Leu-Thr-Ser-Asn-Thr-Lys-Cys-Lys-Glu-Gly-Ser-Arg-Ser-Asn (P15: SEQ ID No. 40 of the Sequence Listing); and

Ser-Ser-Gly-Tyr-Glu-Gly-Gly-Asn-Ile-Tyr-Thr-Lys-Lys-Glu-Ala-Phe-Asn-Val-Glu (P16: SEQ ID No. 41 of the Sequence Listing).

P1 to P15 are partial sequences of the amino acid sequence of Nos. 1 to 157 of the extracellular domain of human Fas, with between 9 and 11 amino acid residues overlapping one another. P16 is a negative control having no homology with human Fas.

P1 to P16 were respectively dissolved completely in 48 µl dimethyl sulfoxide (DMSO) and each was then adjusted to a final volume of 0.8 ml by the addition of 752 µl PBS containing 1 mM  $\beta$ -mercaptoethanol.

The above peptides each correspond to a portion of the extracellular domain of the human Fas molecule, but with a carboxyl group added to the C-terminus. Each peptide was diluted to 50 µg/ml with 0.05 M carbonate-bicarbonate buffer (pH 9.6), containing 10 mM 2-mercaptoethanol, and 50 µl of each were introduced into a well of a 96-well ELISA microplate (Nunc). The plate was kept standing at 4°C overnight to allow adsorption of the peptide to the well surface.

After this time, the solution in the wells was discarded and each well was washed 4 times with PBS-Tween. Then, 100 µl of PBS containing 1% (w/v) bovine serum albumin (A3803; Sigma Chemicals Co.) was added to each well and the plate was incubated at 37°C for 1 hour. The wells were then washed a further 4 times with PBS-Tween, and then 50 µl of HFE7A or CH11 adjusted to 5 µg/ml in PBS was added to each well. The plate was then incubated at 37°C for 1 hour, and the wells were again washed 4 times with PBS-Tween. After washing, 50 µl of horse radish peroxidase labeled goat anti-mouse immunoglobulin antibody (Amersham), diluted 1000-fold with PBS, was added per well, and the plate was again incubated at 37°C for 1 hour, after which the wells were washed 4 times

with PBS-Tween. Horse radish peroxidase substrate (BioRad) was then added in an amount of 100 µl/well and the plate was allowed to stand at room temperature for 15 minutes before reading the absorbance of each well at 415 nm using a microplate reader (Corona). As a positive control, the human Fas fusion protein prepared in Reference Example 1 was used in place of the synthetic peptides.

Using the above methodology, it was established that only the wells with adsorbed P11 showed high absorbance values, demonstrating that HFE7A specifically binds an amino acid sequence contained in P11 (Figure 3).

(6-2) Identification of the epitope recognized by HFE7A in P11 by competitive assay

The following peptides were synthesized:

His-Gly-Leu-Glu-Val-Glu-Ile-Asn-Cys-Thr

(P95: SEQ ID No. 42 of the Sequence Listing);

Glu-Ile-Asn-Cys-Thr-Arg-Thr-Gln-Asn-Thr

(P100: SEQ ID No. 43 of the Sequence Listing);

Arg-Thr-Gln-Asn-Thr-Lys-Cys-Arg-Cys-Lys

(P105: SEQ ID No. 1 of the Sequence Listing);

Lys-Cys-Arg-Cys-Lys-Pro-Asn-Phe-Phe-Cys

(P110: SEQ ID No. 44 of the Sequence Listing);

Pro-Asn-Phe-Phe-Cys-Asn-Ser-Thr-Val-Cys-Glu-His-Cys-Asp

(P115L: SEQ ID No. 45 of the Sequence Listing); and

Gly-Lys-Ile-Ala-Ser-Cys-Leu-Asn-Asp-Asn

(D355-364: SEQ ID No. 46 of the Sequence Listing).

P95, P100, P105 and P110 are each 10-residue partial sequences of the flanking region (corresponding to amino acids 95 to 128 of the extracellular domain of human Fas) of the amino acid sequence

corresponding to P11 in the extracellular domain of human Fas, each having 5 overlapping amino acid residues with the next.

Intended Peptide P115,

Pro-Asn-Phe-Phe-Cys-Asn-Ser-Thr-Val-Cys

(P115: amino acid Nos. 1 to 10 of SEQ ID No. 45 of the Sequence Listing) has a 5-residue overlap with a 10-residue peptide P110, but was expected to have poor solubility, so 4 extra residues were added at the C-terminus of P115 to produce P115L.

D355-364 was used as a negative control, this peptide having no homology with human Fas.

Each peptide, except P115L, was dissolved completely in 16 µl DMSO each was then adjusted to a final volume of 0.8 ml by the addition of 784 µl PBS containing 1 mM 2-mercaptoethanol. P115L was dissolved completely in 48 µl DMSO and was then adjusted to a final volume of 0.8 ml by the addition of PBS containing 1 mM 2-mercaptoethanol.

Each of the above peptide solutions (corresponding to 200 µg peptide) were mixed with 0.25 µg of HFE7A in a microtube and adjusted to a total volume of 100 µl with PBS containing 1 mM 2-mercaptoethanol. The mixture was incubated at 37°C for 2 hours with stirring at 10 to 20 r.p.m., followed by the addition of FCS to a final concentration of 5%, thereby to yield the peptide-antibody mixture.

WR19L12a cells were grown up by a method similar to that described in Reference Example 2. The cells were then recovered by centrifugation and adjusted to a cell density of  $1 \times 10^7$  cells/ml with serum-free RPMI medium. The cell suspension was dispensed into a 96-well plate, with the wells having U-shaped bottoms, at 100 µl/well and centrifuged at 4°C, 1,000 r.p.m. for 3 minutes using a swing rotor for the microplates,

and the supernatant was then discarded. Next, 100 µl of peptide-antibody mixture was added to each pellet and mixed by pipetting a few times, as described above. The plate was then allowed to stand at 4°C for 30 minutes, and was then centrifuged and the supernatant discarded. The pellet was washed 3 times with flow cytometry buffer, and then 50 µl FITC-labeled goat anti-mouse IgG antibody (Kappel), diluted 250-fold with flow cytometry buffer, was added per well, followed by light pipetting to mix the well contents.

The plate was kept in the dark at 4°C for 30 minutes, then centrifuged and the supernatant discarded. The pellet was washed 3 times with flow cytometry buffer, which contained 10% v/v neutral buffered formaldehyde solution (Wako Pure Chemical Industries, Ltd.) for tissue fixation, this solution being 10-fold diluted with PBS and 50 µl/well was added and mixed with light pipetting. Next, the plate was kept in the dark at 4°C for at least 12 hours to fix the cells.

After this time, the cells were suspended in 100 µl/well of flow cytometry buffer and centrifuged, in order to remove the supernatant. The pellet was washed 3 times with flow cytometry buffer and suspended in 500 µl/well of flow cytometry buffer, and the resulting suspension was analyzed with a flow cytometer (Cytoace-150; Nippon Bunko, K. K. - excitation wave length: 488 nm; detection wave length: 530 nm) to calculate average intensities of FITC fluorescence per cell. Average intensities of FITC fluorescence for each sample were calculated by taking the value with no peptide-antibody mixture as 0% and the value of the sample containing D355-364 as 100%.

By the above procedure, it was established that P105 is able to strongly inhibit binding between HFE7A and WR19L12a cells, and that P100 and P110, the amino acid sequence of each of which overlaps 50% with P105, each inhibit binding between HFE7A and WR19L12a cells by about 50%

and 60%, respectively. No inhibition was observed with either of P95 and P115L, which also have no overlapping segments shared with P105 (Figure 4). From these results, it was established that P105 represents an amino acid sequence capable of inhibiting binding between HFE7A and human Fas and that, consequently, the epitope for HFE7A must be located within the amino acid sequence reproduced in P105. This epitopic amino acid sequence is a region which is conserved between human Fas and mouse Fas.

REFERENCE EXAMPLE 7

Binding of HFE7A to Simian Fas

The following test was performed, in order to establish whether HFE7A was able to bind Fas antigen from various primate species.

First, peripheral blood samples were taken from a chimpanzee (Sanwa Kagaku Kenkyujo Kumamoto Primates Park, 40 ml), 20 ml from either a Japanese monkey (*Macaca fuscata*) or from a crab-eating monkey (*Macaca irus*) and 3 ml from a marmoset (of the genus *Hapalidae*). The blood samples had 1 ml of heparin (Novoheparin; Novo) added to them and the samples were then slowly layered over an equal volume of Ficol Paque solution [(Pharmacia) specific gravity: 1.077 for all except the crab-eating monkey, which had a specific gravity of 1.072] and centrifuged at 1,700 r.p.m. for 30 minutes in order to obtain a fraction of peripheral blood mononuclear cells. This mononuclear cell fraction was washed twice with Hanks' balanced salt solution and then suspended in RPMI 1640 medium with 10% v/v FCS to a cell density of  $1 \times 10^6$  cells/ml. Phytohemagglutinin-P (Sigma Chemicals, Co.) was added to the resulting suspension to a final concentration of 5  $\mu\text{g}/\text{ml}$  and the sample incubated at 37°C under 5% v/v CO<sub>2</sub> for 24 hours. After this time, the cells were recovered by centrifugation, washed and resuspended in RPMI 1640 medium

containing 10% v/v FCS. Then, to activate the recovered cells, interleukin-2 (Amersham) was added to the suspension to a final concentration of 10 units/ml, and this was incubated at 37°C under 5% v/v CO<sub>2</sub> for 72 hours.

An amount of the activated preparation calculated to contain  $1 \times 10^6$  activated lymphocyte cells was placed in a test tube and either suspended in 50 µl of 20 µg/ml HFE7A in PBS or 50 µl of PBS alone. The resulting suspension was allowed to stand on ice for 1 hour, after which the cells were washed 3 times with aliquots of 500 µl of PBS and then suspended in 50 µl of 20 µg/ml FITC-labeled anti-mouse IgG antibody (Bioresource) in PBS. This suspension was then placed on ice for 30 minutes, and washed 3 times with aliquots of 500 µl of PBS. Using the cells suspended in 500 µl of PBS as controls, the fluorescence intensities were measured, using a flow cytometer (Cytoace; Nippon Bunko, K. K.).

Distributions of cell numbers by fluorescence intensity were obtained and the proportions of the numbers of the stained cells to those of total cells were calculated. As a result, in the samples without HFE7A, the stained cells constituted less than 3% for all species. However, in the samples treated with HFE7A, at least 17% of the cells were stained, the maximum being 82%. Accordingly, HFE7A is capable of binding a wide range of primate Fas including humans against which HFE7A was originally prepared.

#### REFERENCE EXAMPLE 8

##### Apoptosis-inducing Activity of HFE7A on Murine T cells *in vivo*

Either 500 µl of PBS, alone, or 0.05 or 0.1 mg of HFE7A monoclonal antibody (in 500 µl of PBS) was administered intraperitoneally to the

members of groups of three 6-week old female C3H/HeJ mice (from Japan Clea). The mice were anesthetized with ether, 42 hours post administration, and their thymuses removed. These thymuses were washed with RPMI medium containing 10% v/v FCS, and subsequently disrupted, using a spatula on a mesh (Cell Strainer; Falcon). The disrupted cells (which had passed through the mesh) were washed twice with RPMI 1640 medium containing 10% v/v FCS.

Where washing more than once is referred to in any of the Examples herein, it will be understood that the medium with which the washing is performed is replaced with fresh such medium for each wash, unless otherwise required.

The washed cells obtained above were counted and adjusted to  $1 \times 10^6$  cells in 50  $\mu$ l of RPMI 1640 medium containing 10% v/v FCS. Each of the resulting suspensions was dispensed into a well of a 96-well microplate, the wells having U-shaped bottoms (Nunc) and the plate was then centrifuged (90  $\times$  g, 4°C, 10 minutes).

The supernatants were discarded and then one of the following two fluorescence-labeled antibody solutions in PBS, (a) or (b), was added to each well:

- (a) 10  $\mu$ l of 0.5 mg/ml of FITC-labeled anti-mouse CD95 (Fas) antibody (Jo2; PharMingen), and 10  $\mu$ l of 0.5 mg/ml of phycoerythrin (PE) labeled anti-mouse CD90 antibody (Thy-1.2; Cedarlane; CD90 being a cell surface antigen expressed only on T cells);
- (b) 10  $\mu$ l of 0.5 mg/ml of FITC-labeled anti-mouse CD4 antibody (L3T4; PharMingen), and 10  $\mu$ l of 0.2 mg/ml of PE-labeled anti-mouse CD8 antibody (Ly-2; PharMingen).

After addition of the antibody mixtures, the plate was shaken to mix the contents of the wells and then kept on ice for 1 hour before

centrifuging (90 x g, 4°C, 10 minutes). After discarding the supernatant and washing the wells twice with 100 µl/well of flow cytometry buffer, cells were fixed by adding 50 µl/well of 3.7% v/v formaldehyde solution and were then stood on ice for 10 minutes. After further centrifugation (90 x g, 4°C, 10 minutes) to remove the supernatant, the cell pellets were again washed with 100 µl/well of flow cytometry buffer and suspended in 100 µl/well of flow cytometry buffer. Using the thus obtained cell suspensions from each well as samples, the fluorescence of samples of  $1 \times 10^4$  cells was measured, using a flow cytometer (Epics Elite; Coulter) under the following conditions:  
 excitation wave length: 488 nm;  
 detection wave length: 530 nm (FITC) or 600 nm (PE).

Fluorescence distributions of FITC and PE for the cell populations of each sample could then be prepared. For the samples to which antibody mixture (a) was added, the proportion of the number of cells that were positive for Fas and CD90 (hereinafter referred to as "Fas<sup>+</sup>CD90<sup>+</sup>") relative to the total cell number was calculated. Similarly, for the samples to which antibody mixture (b) was added, the proportion of the number of cells that were positive for CD4 and CD8 (hereinafter referred to as "CD4<sup>+</sup>CD8<sup>+</sup>") or those that were positive for CD4 but negative for CD8 (hereinafter referred to as "CD4<sup>+</sup>CD8<sup>-</sup>") relative to the total cell number was calculated.

The results are shown as percentages in Table 1, below.

Table 1

Cell	Fas <sup>+</sup> CD90 <sup>+</sup>	CD4 <sup>+</sup> CD8 <sup>+</sup>	CD4 <sup>+</sup> CD8 <sup>-</sup>
PBS only	76.2	62.6	11.7
HFE7A 0.05 mg	2.3	1.9	1.2
HFE7A 0.1 mg	1.7	2.8	0.7

Compared with the group to which PBS only was administered, the proportions of T cells expressing Fas ( $Fas^+CD90^+$ ) in the thymus cells of mice from the groups to which HFE7A was administered were remarkably reduced at both doses. Further, the  $CD4^+CD8^+$  and  $CD4^+CD8^-$  cell populations, known for substantial Fas expression, were also markedly reduced in number after HFE7A administration, compared with the PBS only group.

Accordingly, it was deduced that the anti-Fas monoclonal antibody HFE7A had apoptosis-inducing activity, *in vivo*, on Fas-expressing T cells.

#### REFERENCE EXAMPLE 9

##### Effects of HFE7A on an Autoimmune Disease Model

The effects of administration of anti-Fas monoclonal antibody HFE7A on autoimmune disease symptoms were examined using MRL gld/gld mice. These mice carry a mutant of the Fas ligand gene and serve as an animal model of systemic lupus erythematosus-like autoimmune diseases.

18-week old MRL gld/gld mice (from Japan SLC, K. K.), were treated intraperitoneally with a single dose of either 0.2 or 0.5 mg of HFE7A monoclonal antibody prepared in Reference Example 3 (in 500  $\mu$ l of PBS) or with 500  $\mu$ l of PBS alone.

Each test mouse was monitored for swelling of the ankles as a symptom of autoimmune disease. The degree of swelling was evaluated and recorded over time for each group [c.f. Shin Yonehara, (1994), Nikkei Science Bessatsu, 110, 66-77]. The degree of swelling of the ankles was observed to markedly decrease with administration of HFE7A.

The thymuses were removed from the test mice and the proportions of T cells which expressed Fas in the thymuses were determined by the method described in Reference Example 8 above. The results showed that the number of Fas-expressing T cells in the thymuses were significantly reduced after the administration of HFE7A, in accordance with the results of Reference Example 8.

REFERENCE EXAMPLE 10

Hepatotoxicity Testing

BALB/c mice were intraperitoneally administered a single dose of one of the following:

- i) 0.2 mg HFE7A in 500 µl of PBS;
- ii) 0.5 mg HFE7A in 500 µl of PBS;
- iii) 0.1 mg Jo2 (PharMingen) in 500 µl of PBS; and
- iv) 500 µl of PBS alone.

Of the above, Jo2 is a known anti-mouse Fas antibody which has apoptosis-inducing activity. Blood was taken from the posterior aorta of the mice at 8 hours, 24 hours or 72 hours post administration. Blood was taken at 3 hours post administration for the Jo2-treated mice, while they were still alive. All blood was taken under light ether anesthetization. The blood levels of glutamic-oxaloacetic transaminase (GOT) and glutamic-pyruvic transaminase (GPT) were measured for each blood sample, using an automated analyzer (Model 7250; Hitachi Seisakusyo, K. K.) together with the appropriate reagent for the analyzer (Transaminase-HRII; Wako Pure Chemical Industries, Ltd.). As a result, the Jo2-treated group showed rapid elevation of GOT and GPT values after 3 hours, whereas the corresponding values for the groups treated with HFE7A showed little change, as with the group treated with

PBS only (Figure 5). From these results, it was could be established that HFE7A did not induce acute hepatic disorders.

REFERENCE EXAMPLE 11

Effects on Fulminant Hepatitis Model

It is known that, upon intraperitoneal administration of the anti-mouse Fas antibody Jo2, a mouse develops fulminant hepatitis and dies within several hours [c.f. Ogasawara, J., et al., (1993), Nature, 364, 806]. Accordingly, in order to evaluate the effects of HFE7A on hepatic disorders induced by Jo2, the viability of mice was tested by administering HFE7A simultaneously with, or subsequently to, Jo2 administration.

Female, 6 week old BALB/c mice (three mice per group; from Japan SLC) received intraperitoneal administration of an antibody preparation as follows:

- (A) 0.1 mg of Jo2 in 0.5 ml PBS;
- (B) 0.01 mg of Jo2 in 0.5 ml PBS;
- (C) 0.1 mg of Jo2 and 0.5 mg of HFE7A together in 0.5 ml PBS (simultaneous administration);
- (D) 0.1 mg of Jo2 and 0.05 mg of HFE7A in 0.5 ml PBS (simultaneous administration); and
- (E) 0.01 mg of Jo2 in 0.2 ml PBS, followed by 0.1 mg of HFE7A in 0.2 ml PBS after 20 minutes;

and the mice were then observed over time. The results are shown in Figure 6.

When Jo2 alone was administered, all mice died within 9 hours, regardless of whether they were administered with 0.1 mg or with 0.01 mg Jo2/mouse, i.e., mice of groups (A) and (B) above all died within 9

hours of administration. In contrast, when HFE7A was administered simultaneously with Jo2 (both 0.5 mg/mouse and 0.05 mg/mouse), i.e., groups (C) and (D) above, the mice showed no disorders even for several weeks post administration, demonstrating that HFE7A administration can block the development of fulminant hepatitis. Moreover, mice remained normal, with no apparent symptoms developing, even when HFE7A was administered 20 minutes after Jo2 administration.

Thus, HFE7A has preventive and therapeutic effects on various diseases involving disorders of normal tissues which are mediated by the Fas/Fas ligand system, both in the liver and in other organs.

REFERENCE EXAMPLE 12

Effects on Rheumatoid Arthritis

- 1) Preventative effect on the development of collagen-induced arthritis

F1 mice obtained from the mating of a female BALB/c mouse and a male DBA/1J mouse (CD1F1 mice, 6 weeks old, female, from Japan Charles River, K. K.) were tamed for 1 week. After this time, the mice were treated with collagen to induce arthritis.

In more detail, the method was based on one described in the literature [c.f. Phadke, K., (1985), Immunopharmacol., 10, 51-60]. In this method, a 0.3% w/v solution of bovine collagen type II (Collagen Gijutsu Kensyukai, supplied in a 50 mM acetic acid solution) was diluted to 0.2% (2 mg/ml) with further 50 mM acetic acid and then emulsified with an equal volume of Freund's complete adjuvant (Difco). This emulsion was then administered, in an amount of 100 µl (corresponding to 100 µg bovine collagen type II), intradermally in the proximal portion

of the tail, which was held in a fixing device for intravenous injection, using a 1 ml plastic syringe equipped with a tuberculin needle. An identical booster dose was administered under similar conditions, 1 week after the initial challenge.

At the same time as the booster injection, an injection of 100 µg of either HFE7A or control mouse IgG in 0.5 ml PBS was administered intraperitoneally (6 mice per group). Starting five weeks after the initial challenge, swelling of the limbs was monitored visually. The degree of swelling of the joints of the limbs was scored based on the method of Wood, F. D., et al. [Int. Arch. Allergy Appl. Immunol., (1969), 35, 456-467]. Accordingly, the following criteria were used in the calculation of the scores for each of the limbs:

Score

- 0: no symptom;
- 1: swelling and reddening of only one of the small joints, e.g., of a toe;
- 2: swelling and reddening of 2 or more small joints, or of one relatively large joint, such as an ankle; and
- 3: swelling and reddening of a limb in its entirety.

Accordingly, the maximum score for one animal is when all 4 limbs swell, and is 12. An animal scoring at least 1 for all four limbs was designated as an "affected mouse." The results are shown in Figure 7.

In the control group to which non-specific mouse IgG was given, all mice were affected by the 7th week after the initial challenge, whereas in the group to which HFE7A was administered, a half of the mice showed no reddening of any joints at all up to the 8th week (Figure 7A). In addition, the HFE7A-treated group had a lower average score compared with the control group (Figure 7B).

## 2) Apoptosis-induction in synovial cells from rheumatic patients

The effects of HFE7A on the viability of synovial cells from patients with rheumatoid arthritis were evaluated. The method was as described below, using the reducing power of the mitochondria as the index.

Synovial tissue obtained from an affected region of a patient with rheumatoid arthritis was cut into small pieces, with scissors, in Dulbecco's modified Eagle medium (Gibco) supplemented with 10% v/v FCS (Summit). The fat was removed and collagenase (Sigma Chemical Co.) was then added to a final concentration of 5 µg/ml and the mixture was incubated at 37°C for 90 minutes under 5% v/v CO<sub>2</sub>. The resulting incubated cells then served as the synovial cells for the remainder of the Experiment.

The thus obtained synovial cells were separated into single cells by treatment with a 0.05% w/v aqueous trypsin solution at 37°C for 2 minutes, then suspended in Dulbecco's modified Eagle medium containing 10% v/v FCS to a cell density of  $1 \times 10^5$ /ml. This cell suspension was then dispensed into wells of a 96-well plate at  $2 \times 10^4$  cells /200 µl per well, and incubated at 37°C under 5% v/v CO<sub>2</sub> for 6 days. The culture supernatant was discarded and the cells were washed 3 times with Hank's buffer (Gibco). After washing, 200 µl of Dulbecco's modified Eagle medium containing 10% v/v FCS and between 10 and 1,000 ng/ml of HFE7A (serial 10-fold dilutions) were added to each well and the plate further incubated at 37°C under 5% v/v CO<sub>2</sub> for 20 hours. Next, 50 µl of an aqueous solution of 1 mg/ml XTT (2,3-bis[2-methoxy-4-nitro-5-sulfophenyl]-2H-tetrazolium-5-carboxanilide inner salt; Sigma Chemical Co.) and 25 µM PMS (phenazine methosulfate; Sigma Chemical Co.) was added to each well (final concentrations: 250 µg/ml XTT and 5 µM PMS).

After a further 4 hours of incubation at 37°C under 5% v/v CO<sub>2</sub>, the absorbance of each well was read at 450 nm.

The viability of cells in each well was calculated according to the following formula:

$$\text{Viability (\%)} = 100 \times (a-b) / (c-b),$$

wherein "a" is the absorbance of a test well, "b" is the absorbance of a well with no cells, and "c" is the absorbance of a well with no antibody added.

The results are shown in Table 2. HFE7A inhibited the survival of synovial cells from patients with rheumatism in a dose-dependent manner.

Table 2

HFE7A concentration (ng/ml)	Average viability (%)
0	100
10	91
100	77
1000	42

REFERENCE EXAMPLE 13

Designing a Humanized Version of the HFE7A Antibody

(1) Molecular modeling of the variable regions of HFE7A

Molecular modeling of the variable regions of HFE7A was performed by the method generally known as homology modeling [c.f. Methods in Enzymology, 203, 121-153, (1991)].

The primary sequences of variable regions of human immunoglobulin registered in the Protein Data Bank (hereinafter referred to as the "PDB"; Chemistry Department, Building 555, Brookhaven National Laboratory, P.O. Box 5000, Upton, NY 11973-5000, USA), for which X-ray crystallography had been performed, were compared with the framework regions of HFE7A determined above. As a result, 1GGI and 2HFL were selected as having the highest homologies of the three-dimensional structures of the framework regions for the light and heavy chains, respectively. Three-dimensional structures of the framework regions were generated by combining the properties of 1GGI and 2HFL and by calculating the properties of the regions of HFE7A, as described below, to obtain the "framework model".

Using the classification described by Chothia et al., the CDR's of HFE7A could be classified as follows: CDRL<sub>2</sub>, CDRL<sub>3</sub> and CDRH<sub>1</sub> all belong to canonical class 1, while CDRL<sub>1</sub>, CDRH<sub>2</sub> and CDRH<sub>3</sub> do not currently appear to belong to any specific canonical class. The CDR loops of CDRL<sub>2</sub>, CDRL<sub>3</sub>, and CDRH<sub>1</sub> were ascribed the conformations inherent to their respective canonical classes, and then integrated into the framework model. CDRL<sub>1</sub> was assigned the conformation of cluster 15B, in accordance with the classification of Thornton et al. [c.f. J. Mol. Biol., 263, 800-815, (1996)]. For CDRH<sub>2</sub> and CDRH<sub>3</sub>, conformations of sequences with high homologies were selected from the PDB and then these were combined with the results of energy calculations. The conformations of the CDR loops with the highest probabilities were then taken and integrated into the framework model.

Finally, energy calculations were carried out to eliminate undesirable contact between inappropriate atoms, in terms of energy, in order to obtain an overall molecular model of HFE7A. The above procedure was performed using the commercially available common

molecular modeling system, AbM (Oxford Molecular Limited, Inc.), although any other appropriate system could have been used.

For the molecular model obtained, the accuracy of the structure was further evaluated using the software, PROCHECK [J. Appl. Cryst., (1993), 26, 283-291], and the degree of surface exposure of each residue was calculated to determine which surface atoms and groups interacted.

(2) Selection of the acceptors

The subgroups of the light and heavy chains of HFE7A shared identities of 79% with the subgroup κIV and also 79% with the subgroup I, respectively, by comparison with the consensus sequences of the respective subgroups of human antibodies. However, there was no human antibody having a combination of a κIV light chain and a sub-group I heavy chain. Thus, 8E10'CL, which has a light chain of subgroup κIII and a heavy chain of subgroup I, having 72% and 77% sequence identities with the light and heavy chains of HFE7A, respectively, was selected as the single human antibody which had light and heavy chains which both had an identity of greater than 70% with the light and heavy chains of HFE7A.

(3) Selection of donor residues to be grafted onto the acceptors

Using the software, Cameleon (Oxford Molecular Limited, Inc.), the amino acid sequence of each of the light and heavy chains of HFE7A was aligned with that of the relevant chain of 8E10'CL, and humanized sequences of the variable regions were made as described in the following Examples in accordance with the general guidelines set out in a) to e) above. Plasmids were constructed which could serve as recombinant vectors comprising DNA nucleotide sequences encoding humanized anti-human Fas antibodies.

REFERENCE EXAMPLE 14Preparation of DNA Encoding Humanized Light Chain

- (1) Cloning of cDNA encoding a full-length human light chain  
(κ chain)

Prior to humanization of the light chain amino acid sequence of the mouse anti-human Fas antibody HFE7A, cDNA cloning of a human immunoglobulin light chain comprising the constant region was first performed.

1) Synthesis of primers

Separation of cDNA encoding a human light chain was carried out by PCR. For the PCR, the following two primers were synthesized:  
5'-GCGAATTCTG CCTTGACTGA TCAGAGTTTC CTCA-3'  
(HVKII5-4: SEQ ID NO. 47 of the Sequence Listing); and  
5'-GCTCTAGATG AGGTGAAAGA TGAGCTGGAG GA-3'  
(HKCL3-3: SEQ ID No. 48 of the Sequence Listing).

2) Construction of a plasmid containing human  
immunoglobulin light chain cDNA

cDNA encoding a full-length human immunoglobulin light chain was prepared by PCR, inserted into a plasmid and cloned into *E. coli*.

The HL-DNA fragment encoding a full-length human immunoglobulin light chain was prepared under the following conditions:

Composition of the PCR reaction solution:

human lymphocyte cDNA library (Life Technologies), 25 ng;  
oligonucleotide primer HVKII5-4, 50 pmol;  
oligonucleotide primer HKCL3-3, 50 pmol;  
25 mM dNTP cocktail, 10 µl;  
100 mM Tris-HCl buffer (pH 8.5), 10 µl;  
1 M potassium chloride [KCl], 5 µl;  
25 mM magnesium chloride [MgCl<sub>2</sub>], 10 µl;  
Taq DNA polymerase (Perkin Elmer Japan), 1 unit;  
Redistilled water to a total volume of 100 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The thus prepared HL-DNA (human light chain DNA) fragment was inserted into plasmid pCR3DNA using a eukaryote TA Cloning Kit (Invitrogen), following the manufacturer's protocol, and introduced into competent *E. coli* TOP10F' contained in the kit, and following the instructions in the kit. Plasmid pHL15-27 carrying the HL-DNA fragment, i.e., cDNA for a human immunoglobulin light chain, was thereby obtained.

(2) Construction of expression vectors for the light chains of humanized versions of the HFE7A antibody

1) Construction of expression plasmid vectors for humanized HFE7A light chain

Humanization of the amino acid sequence of the light chain of the mouse anti-human Fas antibody HFE7A entailed replacing the 47th amino

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acid (proline) and the 49th amino acid (lysine) from the N-terminus of the amino acid sequence of the light chain (hereinafter referred to as "region I") with alanine and arginine, respectively. Alanine (47) and arginine (49) are conserved in the human light chain ( $\kappa$  chain). Further humanization was also performed, and entailed replacing the 80th amino acid (histidine), the 81st amino acid (proline), the 82nd amino acid (valine), the 84th amino acid (glutamic acid), the 85th amino acid (glutamic acid), the 87th amino acid (alanine) and the 89th amino acid (threonine) (hereinafter referred to as "region II") with serine, arginine, leucine, proline, alanine, phenylalanine and valine, respectively, as these are also conserved in the human light chain ( $\kappa$  chain).

Where both regions I and II were humanized, the sequence was designated as "HH type."

Where only region I was humanized, the sequence was designated as "HM type."

Where neither region was humanized, the sequence was designated as "MM type."

Expression plasmids, respectively carrying these 3 types of humanized light chain amino acid sequences from the anti-human Fas antibody HFE7A, were constructed as follows.

- 2) Synthesis of primers for preparing the variable and constant regions of the light chain of humanized HFE7A

PCR was used to construct the following DNA sequences, each of which comprised one of the HH, HM or MM sequences described above,

together with the constant region of the human immunoglobulin light chain ( $\kappa$  chain):

DNA (SEQ ID No. 49 of the Sequence Listing) encoding the HH type polypeptide chain (SEQ ID No. 50 of the Sequence Listing);

DNA (SEQ ID No. 51 of the Sequence Listing) encoding the HM type polypeptide chain (SEQ ID No. 52 of the Sequence Listing); and

DNA (SEQ ID No. 53 of the Sequence Listing) encoding the MM type polypeptide chain (SEQ ID No. 54 of the Sequence Listing).

The following 13 oligonucleotide PCR primers were synthesized:

5'-CCCAAGCTTA AGAACATCC TCTCATCTAG TTCT-3'

(7AL1P; SEQ ID No. 55);

5'-GAGAGGGTGG CCCTCTCCCC TGGAGACAGA GACAAAGTAC CTGG-3'

(7AL1N; SEQ ID No. 56);

5'-CCAGGTACTT TGTCTCTGTC TCCAGGGAG AGGGCACCCC TCTC-3'

(7AL2P; SEQ ID No. 57);

5'-GATTGAGAT TGGATGCAGC ATAGATGAGG AGTCTGGGTG CCTG-3'

(7AL2N; SEQ ID No. 58);

5'-GCTGCATCCA ATCTCGAAC TGGGATCCC GACAGGTTA GTGGC-3'

(7AL3PA; SEQ ID No. 59);

5'-AAAATCCGCC GGCTCCAGAC GAGAGATGGT GAGGGTGAAG TCTGTCCCAG AC-3'

(7AL3N; SEQ ID No. 60);

5'-CTCGTCTGGA GCCGGCGGAT TTTGCAGTCT ATTACTGTCA GCAAAGTAAT GAGGATCC-3'

(7AL4P; SEQ ID No. 61);

5'-TGAAGACAGA TGGTGCAGCC ACAGTCCGTT TGATTTCCAG CCTGGTGCCT TGACC-3'

(7AL4N; SEQ ID No. 62);

5'-GGTCAAGGCA CCAGGCTGGA AATCAAACGG ACTGTGGCTG CACCATCTGT CTTCA-3'

(7ALCP; SEQ ID No. 63);

5'-CCCGAATTCT TACTAACACT CTCCCTGTT GAAGCTCTTT GTGAC-3'

(7ALCN; SEQ ID No. 64);

5'-TCTGTCCCAG ACCCACTGCC ACTAAACCTG TCTGGGATCC CAGATTCGAG ATTGG-3'

(M7AL2N; SEQ ID No. 65);

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5' -GTTTAGTGGC AGTGGGTCTG GGACAGACTT CACCTCTACC ATCCATCCTG TGGAG-3'

(M7AL3PA; SEQ ID No. 66); and

5' -ATGGTGCAGC CACAGTCCGT TTGATTCCA GCCTGGTGCC TTGACCGAAC GTCCG-3'

(7AL4NA; SEQ ID No. 67).

3) Construction of plasmid p7AL-HH (expression plasmid for  
humanized HH type HFE7A light chain)

The VHH-DNA fragment (SEQ ID No. 49 of the Sequence Listing) encoding the amino acid sequence of SEQ ID No. 50 of the Sequence Listing was prepared by performing 3-stage PCR, and then inserted into a plasmid vector and cloned into *E. coli*.

a) First stage PCR

The outline of the first stage PCR for the preparation of VHH-DNA is shown in Figure 8.

The L7A1-DNA fragment, encoding a secretion signal sequence and a portion of the FRL<sub>1</sub> region altered to contain a Hind III restriction enzyme cleavage site at the 5'-end, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pME-L DNA, 200 ng;

oligonucleotide primer 7AL1P, 80 pmol;

oligonucleotide primer 7AL1N, 80 pmol;

dNTP cocktail, 20 µl;

10×Pfu buffer, 20 µl;

Pfu DNA polymerase (Stratagene), 10 units; and

redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The L7A2-DNA fragment, encoding a portion of the FRL<sub>1</sub>, CDRL<sub>1</sub>, FRL<sub>2</sub> and a portion of the CDRL<sub>2</sub> region, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pME-L DNA, 200 ng;  
oligonucleotide primer 7AL2P, 80 pmol;  
oligonucleotide primer M7AL2N, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The L7A3-DNA fragment, encoding the CDRL<sub>2</sub> and a portion of the FRL<sub>3</sub>, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pME-L DNA, 200 ng;  
oligonucleotide primer 7AL3PA, 80 pmol;  
oligonucleotide primer 7AL3N, 80 pmol;  
dNTP cocktail, 20 µl;

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10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The L7A4-DNA fragment, encoding a portion of the FRL<sub>3</sub>, CDRL<sub>3</sub>, FRL<sub>4</sub> and a portion of the constant region was prepared as follows.

Composition of the PCR reaction solution:

plasmid pME-L DNA, 200 ng;  
oligonucleotide primer 7AL4P, 80 pmol;  
oligonucleotide primer 7AL4N, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The L7A5-DNA fragment, encoding a portion of the FRL<sub>4</sub> and the constant region altered to have an EcoRI restriction enzyme cleavage site at the 3'-end, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pHL15-27 DNA, 200 ng;  
oligonucleotide primer 7ALCP, 80 pmol;  
oligonucleotide primer 7ALCN, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

An equal volume of phenol-chloroform (50% v/v phenol saturated with water, 48% v/v chloroform, 2% v/v isoamyl alcohol) was added to 200 µl of each of the PCR products, and vigorously mixed for 1 minute. After this time, the mixture was centrifuged at 10,000 × g, and the aqueous layer was recovered and mixed with an equal volume of chloroform-isoamyl alcohol (96% v/v chloroform and 4% v/v isoamyl alcohol), which was again vigorously mixed for 1 minute. The resulting mixture was centrifuged at 10,000 × g and the aqueous layer was recovered (the series of steps recited in this paragraph is referred to, herein, as "phenol extraction").

Ethanol precipitation was then performed on the recovered aqueous layer. As used and referred to herein, "ethanol precipitation" consists of adding, with mixing, a one tenth volume of 3M sodium acetate (pH 5.2) and 2.5 volumes of 100% ethanol to the solution to be treated, and freezing the mixture using dry ice. The resulting mixture is then centrifuged at 10,000 × g to recover DNA as a precipitate.

After phenol extraction and ethanol precipitation, the resulting DNA precipitate was vacuum-dried, dissolved in a minimum of redistilled water, and separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with a 1 µg/ml aqueous solution of ethidium bromide to allow detection of DNA under UV light. The DNA bands corresponding to L7A1-DNA, L7A2-DNA, L7A3-DNA, L7A4-DNA and L7A5-DNA were cut out using a razor blade and eluted from the gel using Centrifuter and Centricon-10, as described above. The eluted DNA was then concentrated by centrifugation at 7,500 × g, followed by ethanol precipitation, and finally dissolved in 50 µl of distilled water.

b) Second stage PCR

The outline of the second stage PCR for the production of VHH-DNA is shown in Figure 9.

L7A1.2-DNA, in which the L7A1-DNA and L7A2-DNA fragments, described above, were fused, was prepared as follows.

Composition of the PCR reaction solution:

L7A1-DNA solution prepared in the first stage PCR, 10 µl;  
L7A2-DNA solution prepared in the first stage PCR, 10 µl;  
oligonucleotide primer 7AL1P, 80 pmol;  
oligonucleotide primer 7AL2N, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

L7A4.5-DNA, in which the L7A4-DNA and L7A5-DNA fragments described above were fused, was prepared as follows.

Composition of the reaction solution:

L7A4-DNA solution prepared in the first stage PCR, 10 µl;  
L7A5-DNA solution prepared in the first stage PCR, 10 µl;  
oligonucleotide primer 7AL4P, 80 pmol;  
oligonucleotide primer 7ALCN, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

First, phenol extraction and then ethanol precipitation were performed on the amplified PCR L7A1.2-DNA and L7A4.5-DNA fragments, and these fragments were then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide, and the bands detected under UV light were cut out using a razor blade and eluted from the gel using a Centrifruter and Centricon-10, as described above. The eluted DNA was concentrated

first by centrifugation at 7,500 x g, then by ethanol precipitation, and then dissolved in 50 µl of distilled water.

c) Third stage PCR

The outline of the third stage PCR for the production of VHH-DNA is shown in Figure 10.

The VHH-DNA fragment in which the above described L7A1.2-DNA and L7A4.5-DNA fragments and L7A3-DNA were fused was prepared as follows.

Composition of the PCR reaction solution:

L7A1.2-DNA solution prepared in the second stage PCR, 10 µl;  
L7A4.5-DNA solution prepared in the second stage PCR, 10 µl;  
L7A3-DNA solution prepared in the first stage PCR, 10 µl;  
oligonucleotide primer 7AL1P, 80 pmol;  
oligonucleotide primer 7ALCN, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The amplified PCR VHH-DNA fragment was subjected first to phenol extraction and then to ethanol precipitation, before separation on a 5% w/v polyacrylamide electrophoresis gel. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide and the VHH-DNA band

detected under UV light was cut out using a razor blade and eluted from the gel using a Centrifiruter and Centricon-10, as described above. The eluted DNA was then concentrated by centrifugation at 7,500 x g, followed by ethanol precipitation, and finally dissolved in 50 µl of distilled water.

The construction of an expression plasmid carrying VHH-DNA fragment is outlined in Figure 11.

The VHH-DNA fragment obtained above was further purified by phenol extraction followed by ethanol precipitation, and it was then digested with the restriction enzymes Hind III and EcoRI.

One µg of cloning plasmid pHSG399 DNA (Takara Shuzo Co., Ltd.) was digested with the restriction enzymes Hind III and EcoRI, and then dephosphorylated with alkaline phosphatase (derived from calf intestine; hereinafter abbreviated as CIP). The resulting, dephosphorylated plasmid pHSG399 DNA and the digested VHH-DNA fragment were ligated using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.) using the manufacturer's protocol.

The ligated DNA was recovered by ethanol precipitation, dissolved in 5 µl of redistilled water, and then mixed with *E. coli* JM109 Electro-Cell (Takara Shuzo Co., Ltd.). The mixture was transferred to a Gene Pulser/*E. coli* Pulser Cuvette, 0.1 cm (BioRad) and the ligated mix was then used to transform the *E. coli* JM 109 using Gene Pulser II (BioRad) by the manufacturer's protocol (the series of steps in this paragraph is referred to herein as "transformation").

After transformation, the cells were plated onto LB agar medium [Bacto-tryptone (Difco) 10 g, Bacto-yeast extract (Difco) 5 g, NaCl 10 g, Bacto-agar (Difco) 15g; dissolved in distilled water, q.s. to 1l]

containing final concentrations of 1 mM IPTG (isopropylthio- $\beta$ -D-galactoside; Takara Shuzo Co., Ltd.), 0.1% w/v X-Gal (5-bromo-4-chloro-3-indolyl- $\beta$ -D-galactoside; Takara Shuzo Co., Ltd.) and 50  $\mu$ g/ml chloramphenicol, and the plates were incubated at 37°C overnight to obtain *E. coli* transformants.

Any white transformants obtained were cultured in 2 ml of liquid LB medium at 37°C overnight, and plasmid DNA was extracted from the resulting culture by the alkaline-SDS method [Sambrook, J., et al., (1989), in "Molecular Cloning: A Laboratory Manual (2nd Edition)", Cold Spring Harbor Laboratory Press].

The resulting, extracted plasmid DNA was digested with the restriction enzymes Hind III and EcoRI, and a clone carrying the VHH-DNA fragment was then selected by 1% w/v agarose gel electrophoresis.

Plasmid pHSGHH7 carrying a fusion fragment of the variable region of the humanized HH type HFE7A light chain and DNA encoding the constant region of human immunoglobulin  $\kappa$  chain was obtained accordingly. The transformant *E. coli* pHSGHH7 SANK 73497 harboring plasmid pHSGHH7 was deposited with the Kogyo Gijutsuin Seimei-Kogaku Kogyo Gijutsu Kenkyujo on August 22, 1997, in accordance with the Budapest Treaty, and was accorded the accession number FERM BP-6073.

Using above described plasmid pHSGHH7, it was then possible to construct the expression vector plasmid p7AL-HH, carrying the DNA of SEQ ID No. 49 of the Sequence Listing and which encodes the humanized HH type HFE7A light chain polypeptide of SEQ ID No. 50 of the Sequence Listing.

One  $\mu$ g of pEE.12.1 DNA (Lonza), an expression vector for mammalian cells, was digested with the restriction enzymes Hind III and EcoRI, and

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then dephosphorylated using CIP. The resulting digested, dephosphorylated plasmid DNA (100 ng) was ligated with 10 µg of the pHSGHH7 DNA fragment which had also been digested with Hind III and EcoRI, using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). The ligation mix was then used to transform *E. coli* JM109 (as described above), which was then plated on LB agar plates containing 50 µg/ml ampicillin.

The transformants obtained by this method were cultured in 2 ml of liquid LB medium containing 50 µg/ml ampicillin at 37°C overnight, and plasmid DNA was subsequently extracted from the resulting culture by the alkaline-SDS method.

The extracted plasmid DNA was digested with Hind III and EcoRI, and subjected to 1% w/v agarose gel electrophoresis to confirm the presence or absence of the insert of interest. This enabled the isolation of the plasmid p7AL-HH, which contains a fusion fragment having the variable region of the humanized HH type HFE7A light chain together with DNA encoding the constant region of the human immunoglobulin κ chain. The fusion fragment was found to be located downstream of the cytomegalovirus (CMV) promoter in the correct orientation.

4) Construction of plasmid p7AL-HM (expression plasmid for humanized HM type HFE7A light chain)

The VHM-DNA fragment of SEQ ID No. 51 of the Sequence Listing encoding the amino acid sequence of SEQ ID No. 52 of the Sequence Listing was produced by performing a 3-stage PCR, inserted into a plasmid vector and then cloned into *E. coli*.

## a) First stage PCR

The outline of the first stage PCR for the preparation of the VHM-DNA fragment is shown in Figure 12.

The L7A1-DNA fragment, encoding a secretion signal sequence and a portion of FRL<sub>1</sub> having a Hind III restriction enzyme cleavage site added at the 5'-end, the L7A2-DNA fragment, encoding a portion of FRL<sub>1</sub>, CDRL<sub>1</sub>, FRL<sub>2</sub> and a portion of CDRL<sub>2</sub>, and the L7A5-DNA fragment, encoding a portion of FRL<sub>4</sub> and the constant region having an EcoRI site added at the 3'-end, were used in this process, and were those obtained in the preparation of the VHH-DNA fragment in 2) above.

An ML7A3-DNA fragment, encoding CDRL<sub>2</sub>, FRL<sub>3</sub>, CDRL<sub>3</sub>, FRL<sub>4</sub> and a portion of the constant region, was prepared as follows.

## Composition of the PCR reaction solution:

plasmid pME-L DNA, 200 ng;  
oligonucleotide primer 7AL3PA, 80 pmol;  
oligonucleotide primer 7AL4NA, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The PCR products were subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide and the DNA band detected under UV light, corresponding to ML7A3-DNA, was cut out using a razor blade and eluted from the gel using a Centrifuter and Centricon-10, as described above. The eluted DNA was then concentrated by centrifugation at 7,500 × g followed by ethanol precipitation, and then dissolved in 50 µl of distilled water.

b) Second stage PCR

The outline of the second stage PCR for the preparation of VHM-DNA is shown in Figure 13.

A VHM-DNA fusion fragment comprising the L7A1.2-DNA, the ML7A3-DNA and the L7A5-DNA fragment above was prepared as follows.

Composition of the PCR reaction solution:

L7A1.2-DNA solution prepared in the second stage PCR, 10 µl;  
ML7A3-DNA solution prepared in the first stage PCR, 10 µl;  
L7A5-DNA solution prepared in the first stage PCR, 10 µl;  
oligonucleotide primer 7AL1P, 80 pmol;  
oligonucleotide primer 7ALCN, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30

times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The resulting, amplified VHM-DNA fragment was subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide and the VHM-DNA band thus detected was cut out using a razor blade and eluted from the gel using a Centrifiruter and Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 × g, followed by ethanol precipitation, and dissolved in 50 µl of distilled water.

The construction of an expression plasmid carrying VHM-DNA fragment is outlined in Figure 14.

The VHM-DNA obtained above was further purified by phenol extraction followed by ethanol precipitation, and then digested with the restriction enzymes Hind III and EcoRI.

One µg of the cloning plasmid pHSG399 DNA (Takara Shuzo Co., Ltd.) was digested with the restriction enzymes Hind III and EcoRI, and then dephosphorylated using CIP. The resulting dephosphorylated pHSG399 DNA was then ligated with VHM-DNA, which had also been digested with Hind III and EcoRI, using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). *E. coli* JM109 was then transformed with the ligated DNA and spread onto LB agar medium containing final concentrations of 1 mM IPTG, 0.1% w/v X-Gal and 50 µg/ml chloramphenicol. The white transformants obtained were cultured in 2 ml liquid LB medium containing 50 µg/ml chloramphenicol at 37°C overnight, and plasmid DNA was extracted from the resulting culture by the alkaline-SDS method. The extracted plasmid DNA was then digested with Hind III and EcoRI, and a clone carrying VHM-DNA fragment was selected using 1% w/v agarose gel electrophoresis.

Accordingly, plasmid pHSGHM17, carrying a fusion fragment of the variable region of the humanized HM type HFE7A light chain and DNA encoding the constant region of human Igk chain, was obtained. The transformant *E. coli* pHSGHM17 SANK 73597 harboring plasmid pHSGHM17 was deposited with the Kogyo Gijutsuin Seimeい-Kogaku Kogyo Gijutsu Kenkyujo on August 22, 1997, in accordance with the Budapest Treaty, and was accorded the accession number FERM BP-6072.

Using the above described plasmid pHSGHM17, an expression vector plasmid p7AL-HM was constructed that carried the DNA of SEQ ID No. 51 of the Sequence Listing, encoding the humanized HM type HFE7A light chain polypeptide of SEQ ID No. 52 of the Sequence Listing.

One  $\mu$ g of pEE.12.1 DNA (Lonza), an expression vector for mammalian cells, was digested with the restriction enzymes Hind III and EcoRI, and then dephosphorylated using CIP. The resulting digested, dephosphorylated plasmid DNA (100 ng) was ligated with 10  $\mu$ g of the pHSGHM17-DNA fragment which had also been digested with Hind III and EcoRI, using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). The ligation mix was then used to transform *E. coli* JM109 (as described above), which was then plated on LB agar plates containing 50  $\mu$ g/ml ampicillin.

The transformants obtained by this method were cultured in 2 ml of liquid LB medium containing 50  $\mu$ g/ml ampicillin at 37°C overnight, and plasmid DNA was subsequently extracted from the resulting culture by the alkaline-SDS method.

The extracted plasmid DNA was digested with Hind III and EcoRI, and subjected to 1% w/v agarose gel electrophoresis to confirm the presence or absence of the insert of interest. This enabled the

isolation of the plasmid p7AL-HM, which contains a fusion fragment having the variable region of the humanized HM type HFE7A light chain together with DNA encoding the constant region of the human immunoglobulin κ chain. The fusion fragment was found to be located downstream of the cytomegalovirus (CMV) promoter in the correct orientation.

5) Construction of plasmid p7AL-MM (expression plasmid for humanized MM type HFE7A light chain)

The VMM-DNA fragment of SEQ ID No. 53 of the Sequence Listing encoding the amino acid sequence of SEQ ID No. 54 of the Sequence Listing was produced by performing 3-stage PCR, inserted into a plasmid vector, and then cloned into *E. coli*.

a) First stage PCR

The outline of the first stage PCR for the preparation of VMM-DNA is shown in Figure 15.

The L7A1-DNA fragment, encoding a secretion signal sequence and a portion of FRL<sub>1</sub> and having a HindIII restriction enzyme cleavage site added at the 5'-end, and the L7A5-DNA fragment encoding a portion of FRL<sub>4</sub> and the constant region having an EcoRI restriction site added at the 3'-end, were as obtained in the preparation of the VHH-DNA fragment in (2) above. These fragments were used in the first stage PCR construction of VMM-DNA.

The ML7A2M-DNA fragment, encoding a portion of FRL<sub>1</sub>, CDRL<sub>1</sub>, FRL<sub>2</sub>, CDRL<sub>2</sub> and a portion of FRL<sub>3</sub>, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pME-L DNA, 200 ng;  
oligonucleotide primer 7AL2P, 80 pmol;  
oligonucleotide primer M7AL2N, 80 pmol;  
dNTP cocktail, 20  $\mu$ l;  
10 $\times$ Pfu buffer, 20  $\mu$ l;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200  $\mu$ l.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The ML7A3M-DNA fragment, encoding a portion of FRL<sub>3</sub>, CDRL<sub>3</sub>, FRL<sub>4</sub> and a portion of the constant region, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pME-L DNA, 200 ng;  
oligonucleotide primer M7AL3PA, 80 pmol;  
oligonucleotide primer 7AL4NA, 80 pmol;  
dNTP cocktail, 20  $\mu$ l;  
10 $\times$ Pfu buffer, 20  $\mu$ l;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200  $\mu$ l.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The PCR products were subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide and the DNA bands corresponding to ML7A2M-DNA and ML7A3M-DNA, as detected by UV light, were cut out using a razor blade and eluted from the gel using a Centrifiruter and Centricon-10, as described above. The eluted DNA's were concentrated by centrifugation at 7,500 x g, followed by ethanol precipitation, and dissolved in 50 µl of distilled water.

b) Second stage PCR

The outline of the second stage PCR for the preparation of the VMM-DNA is shown in Figure 16.

The ML7A1.2-DNA fragment, comprising a fusion of the above ML7A1-DNA and ML7A2M-DNA fragments, was prepared as follows.

Composition of the PCR reaction solution:

L7A1-DNA solution prepared in the first stage PCR, 10 µl;  
ML7A2M-DNA solution prepared in the first stage PCR, 10 µl;  
oligonucleotide primer 7AL1P, 80 pmol;  
oligonucleotide primer 7AL2N, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30

times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The resulting, amplified ML7A1.2-DNA fragment was subjected first to phenol extraction and then to ethanol precipitation, and separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide and the fusion-DNA band thus detected was cut out using a razor blade and eluted from the gel using a Centrifuter and Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 × g, followed by ethanol precipitation, and dissolved in 50 µl of distilled water.

c) Third stage PCR

The outline of the third stage PCR for the preparation of the VMM-DNA is shown in Figure 17.

The VMM-DNA fragment, comprising a fusion of the above ML7A1.2-DNA, ML7A3M-DNA and the L7A5-DNA fragment, was prepared as follows.

Composition of the PCR reaction solution:

ML7A1.2-DNA solution prepared in the second stage PCR, 10 µl;  
ML7A3M-DNA solution prepared in the first stage PCR, 10 µl;  
L7A5-DNA solution prepared in the first stage PCR, 10 µl;  
oligonucleotide primer 7AL1P, 80 pmol;  
oligonucleotide primer 7ALCN, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The resulting, amplified VMM-DNA fragment was subjected first to phenol extraction and then to ethanol precipitation, and separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide and the VMM-DNA band thus detected was cut out using a razor blade and eluted from the gel using a Centrifuter and Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 x g, followed by ethanol precipitation, and dissolved in 50 µl of distilled water.

The construction of a plasmid carrying the VMM-DNA fragment is outlined in Figure 18.

The VMM-DNA obtained above was further purified by phenol extraction followed by ethanol precipitation, and then digested with the restriction enzymes Hind III and EcoRI.

One µg of the cloning plasmid pHSG399 DNA (Takara Shuzo Co., Ltd.) was digested with the restriction enzymes Hind III and EcoRI, and then dephosphorylated using CIP. The resulting dephosphorylated pHSG399 DNA was then ligated with VMM-DNA, which had also been digested with Hind III and EcoRI, using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). *E. coli* JM109 was then transformed with the ligated DNA and spread onto LB agar medium containing final concentrations of 1 mM IPTG, 0.1% w/v X-Gal and 50 µg/ml chloramphenicol. The white transformants obtained were cultured in 2 ml liquid LB medium containing 50 µg/ml chloramphenicol at 37°C overnight, and plasmid DNA was extracted from

the resulting culture by the alkaline-SDS method. The extracted plasmid DNA was then digested with Hind III and EcoRI, and a clone carrying VMM-DNA fragment was selected using 1% w/v agarose gel electrophoresis.

Accordingly, plasmid pHSGMM6, carrying a fusion fragment of the variable region of the MM type HFE7A light chain and DNA encoding the constant region of human immunoglobulin  $\kappa$  chain was obtained. The transformant *E. coli* pHSGMM6 SANK 73697 harboring plasmid pHSGMM6 was deposited with the Kogyo Gijutsuin Seimeい-Kogaku Kogyo Gijutsu Kenkyujo on August 22, 1997, in accordance with the Budapest Treaty, and was accorded the accession number FERM BP-6071.

The expression vector plasmid p7AL-MM was constructed using the above described plasmid pHSGMM6, and carries the DNA of SEQ ID No. 53 of the Sequence Listing encoding the MM type HFE7A light chain polypeptide of SEQ ID No. 54 of the Sequence Listing.

One  $\mu$ g of pEE.12.1 DNA (Lonza), an expression vector for mammalian cells, was digested with the restriction enzymes Hind III and EcoRI, and then dephosphorylated using CIP. The resulting digested, dephosphorylated plasmid DNA (100 ng) was ligated with 10  $\mu$ g of the pHSGMM6-DNA fragment which had also been digested with Hind III and EcoRI, using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). The ligation mix was then used to transform *E. coli* JM109 (as described above), which was then plated on LB agar plates containing 50  $\mu$ g/ml ampicillin.

The transformants obtained by this method were cultured in 2 ml of liquid LB medium containing 50  $\mu$ g/ml ampicillin at 37°C overnight, and plasmid DNA was subsequently extracted from the resulting culture by the alkaline-SDS method.

The extracted plasmid DNA was digested with Hind III and EcoRI, and subjected to 1% w/v agarose gel electrophoresis to confirm the presence or absence of the insert of interest. This enabled the isolation of the plasmid p7AL-MM, which contains a fusion fragment having the variable region of the MM type HFE7A light chain together with DNA encoding the constant region of the human immunoglobulin κ chain. The fusion fragment was found to be located downstream of the cytomegalovirus (CMV) promoter in the correct orientation.

#### 6) Verification of the nucleotide sequences

To verify that the DNA inserts of plasmids p7AL-HH, p7AL-HM and p7AL-MM have the desired nucleotide sequences, their DNA inserts were analyzed to determine the nucleotide sequences. The oligonucleotide primers prepared for nucleotide sequencing were as follows:

5'-CCCAAGCTTA AGAACATCC-3' (SP1; SEQ ID No. 68);  
5'-ATCTATGCTG CATCCAATCT-3' (SP2; SEQ ID No. 69);  
5'-GTTGTGTGCC TGCTGAATAA-3' (SP3; SEQ ID No. 70);  
5'-CCCGAATTCT TACTAACACT-3' (SP4; SEQ ID No. 71);  
5'-TTATTTCAGCA GGCACACAAC-3' (SP5; SEQ ID No. 72); and  
5'-AGATTGGATG CAGCATAGAT-3' (SP6; SEQ ID No. 73).

The positions to which each primer binds are shown in Figure 19. The determination of the nucleotide sequences was performed by the dideoxynucleotide chain termination method [Sanger, F. S. et al., (1977), Proc. Natl. Acad. Sci. USA, 74, 5463]. The templates used were the respective plasmid DNA's purified by the alkaline-SDS method and by the cesium chloride method [c.f. Sambrook, J. et al. (1989), in "Molecular Cloning: A Laboratory Manual, Second Edition" Cold Spring Harbor Laboratory Press, for both methods].

More specifically, 3 µg of purified plasmid DNA were dissolved in 13 µl of redistilled water, followed by the addition of 2 µl each of 2 mM EDTA and 2 N NaOH, and the mixture was then allowed to stand at room temperature for 5 minutes. Next, 4 µl of 10 M ammonium acetate solution and 100 µl of 100% ethanol were added and mixed in, and the mixture was placed on dry ice for 10 minutes. After this time, the mixture was centrifuged at 15,000 × g, and the pellet obtained was washed with 80% v/v aqueous ethanol and then vacuum-dried. The resulting, dried DNA was dissolved in 7 µl of redistilled water and used for nucleotide sequencing.

The nucleotide sequencing reaction was performed using a 7-Deaza-Sequenase, Version 2.0 Kit (for dCTP; Amersham). A mixture of 7 µl of the above described plasmid solution, 1 pmol of a primer, which had been synthesized in advance, and 1 µl of reaction buffer (provided with the kit) was made up, and this mixture was then incubated at 65°C for 2 minutes. Subsequently, the DNA was annealed with the primer by gradually cooling to room temperature, followed by labeling with [ $\alpha$ -<sup>32</sup>P]dCTP (Amersham). The reaction product was then subjected to gel electrophoresis on a 5% w/v polyacrylamide gel containing 8 M urea in 1× TBE buffer (100 mM Tris, 100 mM boric acid, 1 mM EDTA, pH8.3). After drying, the sequences on the gel were read by autoradiography. As used herein, all nucleotide sequencing was performed as above, unless otherwise specified.

As a result, it was established that the DNA inserts of plasmids p7AL-HH, p7AL-HM and p7AL-MM had the expected nucleotide sequences, that is:

SEQ ID No. 49 encoding the polypeptide sequence of SEQ ID No. 50; SEQ ID No. 51 encoding the polypeptide sequence of SEQ ID No. 52; and SEQ ID No. 53 encoding the polypeptide sequence of SEQ ID No. 54; respectively, of the Sequence Listing.

REFERENCE EXAMPLE 15Construction of an Expression Vector for the Heavy Chain of the Humanized Version of the HFE7A Antibody(1) Construction of a plasmid carrying the heavy chain variable region DNA of humanized HFE7A1) Synthesis of primers for preparing the variable region of the humanized heavy chain

The synthesis of DNA (SEQ ID No. 74 of the Sequence Listing) encoding a polypeptide chain comprising the variable region of humanized anti-Fas antibody HFE7A heavy chain and the 5 amino acid residues at the N-terminus of the IgG-CH1 region (SEQ ID No. 75 of the Sequence Listing) was performed using a combination of PCR.

The following 8 PCR primers were synthesized as described above:

5'-GGGAAGCTTG GCTTGACCTC ACCATGGGAT GGAGCTGTAT-3'  
(7AH1P; SEQ ID No. 76);

5'-TGAAGCCCCA GGCTTCTTGA CCTCAGCCCC AGACTGCACC AGTTGGAC-3'  
(7AH1NNEW; SEQ ID No. 77);

5'-TCCACTCAAG CCTCTGTCCA GGGGCCTGTT TTACCC-3'  
(7AH2N; SEQ ID No. 78);

5'-GTCTGGGCT GAGGTCAAGA AGCCTGGGC TTCAGTGAAG GTGTCTGCA AG-3'  
(7AH2PNEW; SEQ ID No. 79);

5'-CAGGCCCTG GACAGAGGCT TGAGTGGATG GGAGAGATT-3'  
(7AH3P; SEQ ID No. 80);

5'-TCAGATCTCA GGCTGCTGAG CTCCATGTAG GCTGTGCTAG CGGATGTGTC-3'  
(7AH3N; SEQ ID No. 81);

5'-TGGAGCTCAG CAGCCTGAGA TCTGAGGACA CGGCAGGTCTA TTAC-3'

(7AH4P; SEQ ID No. 82); and

5'-GATGGGCCCT TGGTGGAGGC TGAGGAGACG GTGACCAGGG TCCCTTCGCC CCAGT-3'

(7AH4N; SEQ ID No. 83).

2) Construction of plasmid pBL7A27

The VD-DNA fragment (SEQ ID No. 74 of the Sequence Listing) encoding the amino acid sequence of SEQ ID No. 75 of the Sequence Listing, was prepared by performing 3-stage PCR, then inserted into a plasmid and cloned into *E. coli*.

a) First stage PCR

The outline of the first stage PCR for the preparation of VD-DNA is shown in Figure 20.

The H7A1-DNA fragment, encoding a secretion signal sequence and an N-terminal portion of FRH<sub>1</sub> and having a Hind III restriction enzyme cleavage site added at the 5'-end, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pME-H DNA, 200 ng;

oligonucleotide primer 7AH1P, 80 pmol;

oligonucleotide primer 7AH1NNEW, 80 pmol;

dNTP cocktail, 20 µl;

10×Pfu buffer, 20 µl;

Pfu DNA polymerase, 10 units; and

redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30

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times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The H7A2-DNA fragment, encoding a portion of FRH<sub>1</sub>, CDRH<sub>1</sub>, and a portion of FRH<sub>2</sub>, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pME-H DNA, 200 ng;  
oligonucleotide primer 7AH2N, 80 pmol;  
oligonucleotide primer 7AH2PNEW, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The H7A3-DNA fragment, encoding a portion of FRH<sub>2</sub>, CDRH<sub>2</sub> and a portion of FRH<sub>3</sub>, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pME-H DNA, 200 ng;  
oligonucleotide primer 7AH3P, 80 pmol;  
oligonucleotide primer 7AH3N, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The H7A4-DNA fragment, encoding a portion of FRH<sub>3</sub>, CDRH<sub>3</sub>, FRH<sub>4</sub> and the 5 N-terminal amino acid residues of the CH1 region, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pME-H DNA, 200 ng;  
oligonucleotide primer 7AH4P, 80 pmol;  
oligonucleotide primer 7AH4N, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The respective PCR products were first subjected to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide and the DNA bands corresponding to H7A1-DNA, H7A2-DNA, H7A3-DNA and H7A4-DNA, detected under UV light, were cut out using a razor blade and eluted from the gel

using a Centrifiruter and Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500  $\times$  g, followed by ethanol precipitation, and dissolved in 50  $\mu$ l of distilled water.

b) Second stage PCR

The outline of the second stage PCR for the preparation of VD-DNA is shown in Figure 21.

The H7A1.2-DNA fragment, in which the above described H7A1-DNA and H7A2-DNA fragments were fused, was prepared as follows.

Composition of the PCR reaction solution:

H7A1-DNA solution prepared in the first stage PCR, 10  $\mu$ l;  
H7A2-DNA solution prepared in the first stage PCR, 10  $\mu$ l;  
oligonucleotide primer 7AH1P, 80 pmol;  
oligonucleotide primer 7AH2N, 80 pmol;  
dNTP cocktail, 20  $\mu$ l;  
10 $\times$ Pfu buffer, 20  $\mu$ l;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200  $\mu$ l.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The H7A3.4-DNA fragment, in which the above described H7A3-DNA and H7A4-DNA fragments were fused, was prepared as follows.

Composition of the PCR reaction solution:

H7A3-DNA solution prepared in the first stage PCR, 10 µl;  
H7A4-DNA solution prepared in the first stage PCR, 10 µl;  
oligonucleotide primer 7AH3P, 80 pmol;  
oligonucleotide primer 7AH4N, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The resulting, amplified H7A1.2-DNA and H7A3.4-DNA fragments were subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gels were stained with 1 µg/ml of ethidium bromide and the relevant bands thus detected were cut out using a razor blade and eluted from the gel using a Centrifiruter and Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 × g, followed by ethanol precipitation, and dissolved in 50 µl of distilled water.

c) Third stage PCR

The outline of the third stage PCR for the preparation of VD-DNA is shown in Figure 22.

The VD-DNA fragment, in which above described H7A1.2-DNA and H7A3.4-DNA fragments were fused, was prepared as follows.

Composition of the PCR reaction solution:

H7A1.2-DNA solution prepared in the second stage PCR, 10 µl;  
H7A3.4-DNA solution prepared in the second stage PCR, 10 µl;  
oligonucleotide primer 7AH1P, 80 pmol;  
oligonucleotide primer 7AH4N, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The resulting, amplified VD-DNA fragment was subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide and the VD-DNA band thus detected was cut out using a razor blade and eluted from the gel using a Centrifuter and Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 x g, followed by ethanol precipitation, and dissolved in 50 µl of distilled water.

The construction of a plasmid carrying the VD-DNA fragment is outlined in Figure 23.

The VD-DNA fragment obtained above was further purified by phenol extraction followed by ethanol precipitation, and then digested with the restriction enzymes Hind III and ApaI.

One µg of the plasmid vector pBLUESCRIPT-II SK+ DNA (Stratagene) was digested with Hind III and Apa I, and then dephosphorylated using CIP. The resulting, dephosphorylated plasmid DNA and 100 ng of the VD-DNA fragment, which had also been digested with Hind III and Apa I, were ligated using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). The resulting ligation mix was then used to transform *E. coli* JM109, which was then plated on LB agar plates containing final concentrations of 1 mM IPTG, 0.1% w/v X-Gal and 50 µg/ml ampicillin. Any resulting white transformants were cultured in 2 ml liquid LB medium containing 50 µg/ml ampicillin at 37°C overnight, and plasmid DNA was then extracted from the culture by the alkaline-SDS method. The resulting plasmid was digested with Hind III and Apa I and subjected to agarose gel electrophoresis to confirm the presence or absence of the insert of interest. Accordingly, the plasmid pBL7A27 with a VD-DNA insert was obtained.

(2) Construction of a plasmid carrying human IgG1 constant region genomic DNA

1) Synthesis of primers for preparing 5'-terminal human IgG1 genomic DNA fragment

A 5'-terminal human IgG1 genomic DNA fragment was synthesized by PCR. For this, the following 2 oligonucleotide primers were prepared:

5'-GGGAAGCTTC CGCGGTACCA TGGCACCCACC TCTCTTGCA-3'

(5'Hind: SEQ ID No. 84 of the Sequence Listing); and

5'-GCTCTGCAGA GAGAAGATTG GGAGTTACTG GAATC-3'

(IGGCPSTN: SEQ ID No. 85 of the Sequence Listing).

2) Construction of plasmid pIG5'03

Genomic DNA, comprising the CH1 region of human IgG1 together with an intron following a Hind III cleavage sequence, was separated and amplified by PCR using human genomic DNA as the template, and then inserted into the plasmid pHSG399 (Takara Shuzo Co., Ltd.) and cloned into *E. coli*. The preparation of this DNA (hereinafter referred to as "IG5'-DNA") is outlined in Figure 24.

An IG5'-DNA fragment was prepared as follows.

Composition of the PCR reaction solution:

human genomic DNA (Clonetech), 2 µg;  
oligonucleotide primer 5'Hind, 80 pmol;  
oligonucleotide primer IGGCPSTN, 80 pmol;  
dNTP cocktail, 20 µl;  
10×Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The resulting, amplified IG5'-DNA fragment was subjected first to phenol extraction and then to ethanol precipitation, and separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide and the IG5'-DNA band thus detected was cut out using a razor blade and eluted from the gel using a

Centrifiruter and Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 × g, followed by ethanol precipitation, and dissolved in 50 µl of distilled water.

The IG5'-DNA fragment thus obtained was further purified by phenol extraction and then ethanol precipitation, and was then digested with the restriction enzymes Hind III and Pst I.

One µg of plasmid pHSG399 DNA (Takara Shuzo Co., Ltd.) was digested with the restriction enzymes Hind III and Pst I, and then dephosphorylated using CIP. The resulting dephosphorylated plasmid DNA and 100 ng of the IG5'-DNA fragment, which had also been digested with Hind III and Pst I, were ligated using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). The ligation mix was then used to transform *E. coli* JM109, which was then plated onto LB agar medium containing final concentrations of 1 mM IPTG, 0.1% w/v X-Gal and 50 µg/ml chloramphenicol. Any white transformants obtained were cultured in 2 ml liquid LB medium containing 50 µg/ml chloramphenicol at 37°C overnight, and plasmid DNA was extracted from the resulting culture by the alkaline-SDS method. The extracted plasmid DNA was then digested with Hind III and Pst I, and subjected to 1% w/v agarose gel electrophoresis to confirm the presence or absence of the insert of interest. Accordingly, the plasmid pIG5'03, containing a IG5'-DNA fragment insert, was obtained.

(3) Construction of a plasmid carrying human IgG1 constant region genomic DNA

1) Synthesis of primers for preparing 3'-terminal human IgG1 genomic DNA fragment

A 3'-terminal human IgG1 genomic DNA fragment was synthesized by PCR. For this, the following 2 primers were prepared:

5'-TCTCTGCAGA GCCCAAATCT TGTGACAAAA CTCAC-3'

(IGGCPTSP: SEQ ID No. 86 of the Sequence Listing); and

5'-GGGGAATTCTG GGAGCGGGGC TTGCCGGCCG TCGCACTCA-3'

(Eco3': SEQ ID No. 87 of the Sequence Listing).

2) Construction of plasmid pIG3'08

DNA comprising the sequence: intron from human IgG1; hinge region; intron from human IgG1; CH2 region; intron from human IgG1; CH3 region; and an EcoRI cleavage sequence, was separated and amplified by PCR using human genomic DNA as the template, and then inserted into the plasmid pHSG399 (Takara Shuzo Co., Ltd.) and cloned into *E. coli*. The preparation of the above DNA (hereinafter referred to as "IG3'-DNA") is outlined in Figure 25.

IG3'-DNA was prepared as follows.

Composition of the PCR reaction solution:

human genomic DNA (Clonetech), 2 µg;

oligonucleotide primer IGGCPSTP, 80 pmol;

oligonucleotide primer Eco3', 80 pmol;

dNTP cocktail, 20 µl;

10×Pfu buffer, 20 µl;

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Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200  $\mu$ l.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The resulting, amplified IG3'-DNA fragment was subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1  $\mu$ g/ml of ethidium bromide and the IG3'-DNA band thus detected was cut out using a razor blade and eluted from the gel using a Centrifiruter and Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500  $\times$  g, followed by ethanol precipitation, and dissolved in 50  $\mu$ l of distilled water.

The IG3'-DNA fragment thus obtained was further purified by phenol extraction and then ethanol precipitation, and was then digested with the restriction enzymes EcoRI and Pst I.

One  $\mu$ g of plasmid pHSG399 DNA (Takara Shuzo Co., Ltd.) was digested with EcoRI and Pst I, and then dephosphorylated using CIP. The resulting dephosphorylated plasmid DNA was then ligated with 100 ng of the IG3'-DNA fragment, which had also been digested with EcoRI and Pst I, using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). The ligation mix was then used to transform *E. coli* JM109, which was plated onto LB agar medium containing final concentrations of 1 mM IPTG, 0.1% w/v X-Gal and 50  $\mu$ g/ml chloramphenicol. Any white colonies were selected and the plasmid pIG3'08, containing an IG3'-DNA insert, was obtained.

(4) Construction of expression vector plasmid for humanized HFE7A heavy chain

The expression plasmid vector pEg7AH-H, carrying the DNA of SEQ ID No. 88 of the Sequence Listing and encoding the humanized HFE7A heavy chain polypeptide of SEQ ID No. 89 of the Sequence Listing, was constructed using the above described plasmids pBL7A27, pIG5'03 and pIG3'08. The procedure is outlined in Figure 26.

Ten µg of plasmid pIG5'03 DNA, comprising the CH1 region of human IgG1 heavy chain and an intron, was digested with the restriction enzymes ApaI and Kpn I. In addition, 1 µg of pBL7A27 DNA above was also digested with the restriction enzymes Apa I and Kpn I, and then dephosphorylated using CIP. The resulting dephosphorylated pBL7A27 DNA (100 ng) was ligated with 10 µg of the digested and dephosphorylated pIG5'03 DNA, using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). The ligation mix was then used to transform *E. coli* JM109, which was plated on LB medium containing 50 µg/ml ampicillin. Resulting transformants were cultured in 2 ml liquid LB medium containing 50 µg/ml ampicillin at 37°C overnight, and plasmid DNA was extracted from the culture by the alkaline-SDS method. The plasmid was digested with Apa I and Kpn I, or with Hind III and Pst I, to confirm the presence or absence of the insert of interest by 1% w/v agarose gel electrophoresis. Thus, the plasmid pBL7AF184, containing a VD-DNA fragment of humanized HFE7A connected with the IG5'-DNA fragment, was obtained.

Next, 10 µg of the thus obtained plasmid pBL7AF184 was digested with the restriction enzymes Hind III and Pst I, and 1 µg of the plasmid pIG3'08 DNA above was likewise digested with Hind III and Pst I, and dephosphorylated using CIP. The resulting dephosphorylated pIG3'08 DNA (100 ng) was ligated with 10 µg of the digested pBL7AF184 DNA, using a

DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). *E. coli* JM109 was transformed with the ligation mix and plated onto LB medium containing 50 µg/ml ampicillin. Any resulting transformants were cultured in 2 ml liquid LB medium containing 50 µg/ml chloramphenicol at 37°C overnight, and plasmid DNA was extracted from the culture by the alkaline-SDS method. The plasmid was digested with Hind III and Pst I, or with Hind III and EcoRI, to confirm the presence or absence of the insert of interest by 1% w/v agarose gel electrophoresis.

Plasmid pgHSL7A62, containing a VD-DNA fragment of humanized HFE7A connected to a genomic DNA fragment encoding human IgG1 constant region, was obtained. The transformant *E. coli* pgHSL7A62 SANK 73397 harboring plasmid pgHSL7A62 was deposited with the Kogyo Gijutsuin Seimei-Kogaku Kogyo Gijutsu Kenkyujo on August 22, 1997, in accordance with the Budapest Treaty, and was accorded the accession number FERM BP-6074.

Ten micrograms of the thus obtained plasmid pgHSL7A62 DNA were digested with the restriction enzymes Hind III and EcoRI and, likewise, 1 µg of the expression plasmid pEE.6.1 DNA was digested with Hind III and EcoRI, and dephosphorylated using CIP. The resulting dephosphorylated pEE.6.1 DNA (100 ng) was ligated with 10 µg of the digested pgHSL7A62 DNA, using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). *E. coli* JM109 was transformed with the ligation mix and plated onto LB medium containing 50 µg/ml ampicillin. Any resulting transformants were cultured in 2 ml liquid LB medium containing 50 µg/ml ampicillin at 37°C overnight, and plasmid DNA was extracted from the culture by the alkaline-SDS method. The plasmid was digested with Hind III and EcoRI, to confirm the presence or absence of the insert of interest by 1% w/v agarose gel electrophoresis.

The resulting plasmid, pEg7AH-H, contained a fusion fragment comprising a VD-DNA fragment of humanized HFE7A and a genomic DNA fragment encoding human IgG1 constant region in connection and inserted downstream of the CMV promoter in the correct orientation.

(5) Verification of nucleotide sequence

To verify that the DNA insert of the pEg7AH-H had the expected nucleotide sequence, the DNA insert was analyzed to determine the nucleotide sequence. For this, the following primers were synthesized:

5'-ACAGCCGGGA AGGTGTGCAC-3' (IG01: SEQ ID No. 90);  
5'-AGACACCCTC CCTCCCTGTG-3' (IG02: SEQ ID No. 91);  
5'-GTGCAGGGCC TGGGTTAGGG-3' (IG03: SEQ ID No. 92);  
5'-GCACGGTGGG CATGTGTGAG-3' (IG04: SEQ ID No. 93);  
5'-GTTTTGGGGG GAAGAGGAAG-3' (IG05: SEQ ID No. 94);  
5'-CCAGTCCTGG TGCAGGACGG-3' (IG06: SEQ ID No. 95);  
5'-CCTGTGGTTC TCAGGGCTGC-3' (IG07: SEQ ID No. 96);  
5'-CGTGGTCTTG TAGTTGTTCT-3' (IG08: SEQ ID No. 97);  
5'-CTTCCTCTTC CCCCCAAAAC-3' (IGP5: SEQ ID No. 98);  
5'-CCGTCTTGCA CCAGGACTGG-3' (IGP6: SEQ ID No. 99);  
5'-GCAGCCCCGA GAACCACAGG-3' (IGP7: SEQ ID No. 100);  
5'-AGAACAACTA CAAGACCACG-3' (IGP8: SEQ ID No. 101);  
5'-GCCTGACATC TGAGGACTC-3' (H5+: SEQ ID No. 102);  
5'-GAGTCCTCAG ATGTCAGGC-3' (H5-: SEQ ID No. 103);  
5'-GAGCAGTACT CGTTGCTGCC GCGCGCGCCA CCAG-3'  
(PEEF: SEQ ID No. 104); and  
5'-GGTATGGCTG ATTAATGATC AATG-3' (PEEB: SEQ ID No. 105).

The positions to which each primer binds are shown in Figure 27. The determination of the nucleotide sequence was performed by the dideoxynucleotide chain termination method (*ibid.*) using, as templates, the respective plasmids purified by the alkaline-SDS method and the

cesium chloride method (*ibid.*). It was confirmed that pEg7AH-H had the nucleotide sequence of SEQ ID No. 88 of the Sequence Listing, encoding the polypeptide of SEQ ID No. 89 of the Sequence Listing.

REFERENCE EXAMPLE 16

Expression in COS-1 Cells

COS-1 cells (derived from a monkey kidney) were transfected with the expression plasmids for the humanized HFE7A heavy chain and with the expression plasmids for each of the humanized HFE7A light chains obtained above. Transfection was performed by electroporation using the gene transfection apparatus GTE-1 (Shimadzu Seisakusyo, K. K.) equipped with an FCT-13 chamber having electrodes separated by 2 mm (Shimadzu Seisakusyo, K. K.).

COS-1 cells (American Type Culture Collection No. CRL-1650) were grown to semi-confluence in a culture flask (culture area: 225 cm<sup>2</sup>; Sumitomo Bakelite) containing Minimal Essential α medium ["α(+)MEM"; Gibco BRL] supplemented with 10% v/v FCS (Moregate). Subsequently, the medium was discarded and the COS-1 cells were detached from the flask by treatment with 3 ml of trypsin-EDTA solution (Sigma Chemicals Co.) at 37°C for 3 minutes. The detached cells were then harvested by centrifugation at 800 r.p.m. for 2 minutes, discarding the supernatant and washing twice with phosphate buffer (0.02% w/v potassium chloride [KCl], 0.02% w/v potassium dihydrogenphosphate [KH<sub>2</sub>PO<sub>4</sub>], 0.8% w/v sodium chloride [NaCl], 1.15% w/v disodium hydrogenphosphate [Na<sub>2</sub>HPO<sub>4</sub>] ; hereinafter referred to as "PBS(-) buffer"; Nissui Pharmaceutical Co., Ltd.). The washed COS-1 cells were then suspended to a cell density of 1 × 10<sup>8</sup> cells/ml in PBS(-) buffer.

In parallel, 4 µg of humanized HFE7A heavy chain expression plasmid DNA were mixed with 4 µg of humanized HFE7A light chain

expression plasmid DNA, each purified by the alkaline-SDS method and cesium chloride density gradient centrifugation. The resulting mixture was subjected to ethanol precipitation and then suspended in 20 µl of PBS(-) buffer. These mixing, precipitation and resuspension steps were all performed in the same tube. The whole of the resulting plasmid suspension (20 µl) was mixed with 20 µl of the previously prepared COS-1 cell suspension ( $2 \times 10^6$  cells) and the mixture was transferred to an FCT-13 electroporation chamber (Shimadzu Seisakusyo, K. K.) having electrodes set 2 mm apart, which was then loaded into gene transfection apparatus GTE-1 (Shimadzu Seisakusyo, K. K.). Pulses of 600 V, each of 50 µF were applied twice with a 1 second interval, in order transform the COS-1 cells with the plasmid DNA. After electroporation, the cell-DNA mixture in the chamber was suspended in 20 µl of α(+)MEM supplemented with 10% v/v FCS and transferred to a culture flask (culture area  $75 \text{ cm}^2$ ; Sumitomo Bakelite). After incubating under 5% v/v CO<sub>2</sub> at 37°C for 72 hours, the culture supernatant was recovered, and analysis was performed on the supernatants to determine what expression products were present.

Using the above method, but modified as appropriate, COS-1 cells were variously transfected with each of the following plasmid or plasmid combinations:

- (A) : no plasmid DNA
- (B) : cotransfection with pEG7AH-H and p7AL-MM
- (C) : cotransfection with pEG7AH-H and p7AL-HM
- (D) : cotransfection with pEG7AH-H and p7AL-HH

REFERENCE EXAMPLE 17Quantification of Expression Products by ELISA

Verification and quantitative assay of the expression of humanized antibodies as expression products in the culture supernatant fluids prepared in Reference Example 16 were performed by ELISA, using an anti-human IgG antibody.

Goat anti-human IgG Fc specific polyclonal antibody (Kappel) was dissolved to a final concentration of 1 µg/ml in adsorption buffer (0.05 M sodium hydrogencarbonate, 0.02% w/v sodium azide, pH 9.6) and 100 µl aliquots were added to each well of a 96-well plate (MaxiSorb, Nunc), and the plate was incubated at 37°C for 2 hours to encourage adsorption of the antibody. Next, each well was washed with 350 µl of PBS-T [PBS(-) containing 0.05% w/v Tween-20 (BioRad)] four times. After washing, culture supernatant diluted with α(+)MEM containing 10% v/v FCS was added to the wells, and the plate was further incubated at 37°C for 2 hours.

After this time, the wells were again washed four times with PBS-T, and then 100 µl of alkaline phosphatase-labeled goat anti-human IgG Fc specific polyclonal antibody (Caltag Lab.) diluted 5,000-fold with PBS-T were added to each well and the plate was incubated at 37°C for 2 hours. Each well was then again washed four times with PBS-T, and 100 µl of a substrate solution of 1 mg/ml p-nitrophenyl phosphate, prepared in 10% v/v diethanol amine (pH 9.8), was added to each well. After a subsequent incubation at 37°C for 0.5 to 1 hour, absorbance at 405 nm was measured.

In the present experiments, human plasma IgG subclass 1 (IgG1; Biopure AG) diluted with α(+)MEM containing 10% v/v FCS to certain desired concentrations was used to provide concentration reference

samples of the humanized HFE7A antibodies contained in the culture supernatant fluids.

As expected, each supernatants of transformants (B), (C) and (D) was determined to express human antibody, as detected by anti-human IgG antibody. The negative control, (A), showed no expression of human antibody.

REFERENCE EXAMPLE 18

Assay for Fas-Binding Activity

The assay for Fas-binding activity in the cell culture supernatants prepared in Reference Example 16 was performed by ELISA as follows.

Culture supernatant from COS-1 cells expressing the human Fas fusion protein, as obtained in Reference Example 1 above, diluted 5-fold with adsorption buffer, was dispensed into wells of a 96-well plate (MaxiSorb; Nunc) at 50 µl per well and the plate was incubated at 4°C overnight to allow adsorption of the human Fas fusion protein to the surface of the wells. Next, each of the wells was washed 4 times with 350 µl of PBS-T. After washing, PBS-T containing 5% v/v BSA (bovine serum albumin; Wako Pure Chemical Industries, Ltd.) was added to the wells at 50 µl per well and the plate was incubated at 37°C for 1 hour to block the remainder of the surface of each well. The wells were then again washed four times with PBS-T.

The culture supernatants obtained in Reference Example 16 were adjusted to have a final concentration of the product of interest of 100 ng/ml in α(+)MEM containing 10% v/v FCS. Concentrations were estimated by the method described in Reference Example 17. Each of the

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resulting 100 ng/ml solutions was then used to produce serial dilutions by serial 2-fold dilution with α(+)MEM containing 10% v/v FCS. Next, 50 µl of each of the resulting serial dilutions of each expression product was added to a well prepared as above, and the plate was incubated at 37°C for 2 hours to allow reaction.

After this time, the wells were again washed four times with PBS-T, and then 50 µl of alkaline phosphatase-labeled goat anti-human IgG Fc specific polyclonal antibody (Caltag Lab.), diluted 10,000-fold with PBS-T, were dispensed into each well and reaction was allowed to proceed at 37°C for 2 hours.

HFE7A purified from mouse hybridoma HFE7A was used as a control (IgG1), and was detected using alkaline phosphatase-labeled goat anti-mouse IgG + IgA + IgM (Gibco BRL), diluted 5,000-fold with PBS-T, in place of the alkaline phosphatase-labeled goat anti-human IgG Fc specific polyclonal antibody.

The wells were again washed four times with PBS-T, and then 50 µl of substrate solution [1 mg/ml p-nitrophenyl phosphate in 10% v/v diethanol amine (pH9.8)] was dispensed into each well and the plate was incubated at 37°C for 0.5 to 1 hour. Binding activity of the expression product contained in each culture supernatant fluid with the human Fas fusion protein was evaluated by reading the absorbance of each well at 405 nm.

As expected, binding activity to the human Fas fusion protein was demonstrated for supernatants (B), (C) and (D) above (Figure 28).

REFERENCE EXAMPLE 19Competitively Inhibiting Binding of HFE7A to Fas

The humanized anti-Fas antibodies of the Examples should inhibit the binding of HFE7A to Fas, as the antibodies of the Examples were derived from HFE7A. Therefore, the ability of the expression products obtained in Reference Example 16 to competitively inhibit the binding of HFE7A to the human Fas fusion protein was measured.

One mg of the purified monoclonal antibody HFE7A obtained in Reference Example 3 was labeled using a commercially available alkaline phosphatase labeling kit (Immuno-Link AP and APL Labeling Kit; Genosis), using the protocol supplied with the kit. The resulting, labeled antibody is also referred to herein as "AP-HFE7A".

The COS-1 cell culture supernatant containing the human Fas fusion protein, as obtained in Reference Example 1, was diluted 5-fold with adsorption buffer, and dispensed into the wells of a 96-well plate for luminescence detection (Luminescent Solid Assay Plate, high binding property; Costar) at 50 µl per well. The plate was then incubated at 4°C overnight to allow adsorption of the human Fas fusion protein to the surface of the wells.

After this time, each well was washed 4 times with 350 µl of PBS-T, and then 100 µl PBS-T containing 5% v/v BSA was added to each well and the plate was incubated at 37°C for 1 hour to block the remainder of the surface of each well. The wells were then again washed four times with PBS-T.

The culture supernatants obtained in Reference Example 16 were adjusted to final concentrations of antibody of 1 µg/ml in α(+)MEM containing 10% v/v FCS by the method of Reference Example 17. Each of

the resulting solutions of the expression products was used to produce serial dilutions by serial 2-fold dilution with α(+)MEM containing 10% v/v FCS. AP-HFE7A was diluted to 50 ng/ml with α(+)MEM containing 10% v/v FCS, and 25 µl of the resulting solution was mixed with an equal volume of each of the prepared serial dilutions.

Each of the wells was again washed four times with PBS-T, and then 50 µl of each of the resulting antibody mixtures were added to individual wells, and the plate was allowed to stand at room temperature overnight. Subsequently, after washing each well with PBS-T again four times, 100 µl of CDP-star buffer (9.58 ml diethanol amine, 0.2 g magnesium chloride, 0.25 g sodium azide, pH8.5) was dispensed into each well and the plate was allowed to stand at room temperature for 10 minutes. After this time, the CDP-star buffer was discarded and CDP-star substrate [1.2 ml sapphire II (Tropix), 200 µl CDP-star (Tropix), q.s. to 12 ml with CDP-star buffer] was added at 50 µl per well, and the plate was then allowed to stand at room temperature for a further 40 minutes.

Competitive inhibition of the expression products of Reference Example 16 of the binding of HFE7A to the human Fas fusion protein was evaluated by measuring the intensity of the luminescence with Luminoscan (Titertech).

As a result, it was verified that the expression products prepared in Reference Example 16 specifically inhibited the binding of HFE7A to the human Fas fusion protein (Figure 29).

REFERENCE EXAMPLE 20Apoptosis-Inducing Activity

WR19L12a cells (c.f. Itoh, N. et al., *ibid.*) were used to examine the apoptosis-inducing activity of the COS-1 cell culture supernatant of Reference Example 16.

WR19L12a cells were cultured in RPMI 1640 medium with 10% v/v FCS (Gibco BRL) at 37°C for 3 days under 5% v/v CO<sub>2</sub>, and 50 µl (1 × 10<sup>5</sup> cells) of the resulting culture were then dispensed into each well of a 96-well microplate (Sumitomo Bakelite). The culture supernatants obtained in Reference Example 16 were adjusted to a final concentration of antibody of 100 ng/ml in RPMI 1640 medium containing 10% v/v FCS. Concentrations were estimated by the method of Reference Example 17. Each of the adjusted solutions of the expression products was used to produce serial dilutions by serial 2-fold dilution with RPMI 1640 containing 10% v/v FCS. Each of the resulting dilutions of each expression product solution was added to individual wells, at 50 µl per well, and the plate was incubated at 37°C for 1 hour. After this time, the cells in each well were washed four times with RPMI 1640 containing 10% v/v FCS and then the washed cells were suspended in 75 µl per well of RPMI 1640 containing 10% v/v FCS.

Subsequently, 75 µl of 1.25 µg/ml goat anti-human IgG Fc specific polyclonal antibody (Kappel) in RPMI 1640 medium containing 10% v/v FCS was added to each well, as secondary antibody. The plate was allowed to stand at 37°C for 12 hours, and then 50 µl of 25 µM PMS (phenazine methosulfate; Sigma Chemical Co.), containing 1 mg/ml XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxyanilide inner salt; Sigma Chemical Co.] to final concentrations of 250 µg/ml for XTT and 5 µM for PMS, were added to each well. The plate was then incubated for 3 hours at 37°C, and the absorbance at 450 nm of each well was

measured, to calculate cell viability, using the reducing power of the mitochondria as the index.

The viability of the cells in each well was calculated according to the following formula:

$$\text{Viability (\%)} = 100 \times (a-b) / (c-b)$$

wherein "a" is the absorbance of a test well, "b" is the absorbance of a well with no cells, and "c" is the absorbance of a well with no antibody added.

As expected, each of the expression products (B), (C) and (D) of Reference Example 16, were demonstrated to induce apoptosis in T cells expressing the human Fas antigen (Figure 30).

#### REFERENCE EXAMPLE 21

##### Preparation of DNA Encoding Humanized Light Chain

- (1) Construction of vectors for the light chains of humanized versions of HFE7A antibody

In order to humanize the amino acid sequence of the light chain of the mouse anti-human Fas antibody HFE7A, the 1st amino acid (aspartic acid), the 85th amino acid (alanine) and the 107th amino acid (arginine) from the N-terminus of the amino acid sequence of the HH type light chain were replaced with glutamic acid, glutamic acid and lysine, respectively. These replacement residues are conserved in the human light chain ( $\kappa$  chain). The resulting sequence was designated as "PDHH type." For the HM light chain sequence, the 1st amino acid (aspartic acid) and the 107th amino acid (arginine) from the N-terminus of the amino acid sequence were replaced with the conserved glutamic acid and

lysine residues, respectively. The resulting sequence was designated as "PDHM type."

Expression plasmids separately carrying these 2 types of humanized light chain amino acid sequences (PDHH and PFHM) were constructed as follows.

1) Synthesis of primers for preparing the variable and constant regions of the light chain of humanized HFE7A

DNA (SEQ ID No. 106 of the Sequence Listing) encoding the PDHH type polypeptide chain (SEQ ID No. 107 of the Sequence Listing) and DNA (SEQ ID No. 108 of the Sequence Listing) encoding the PDHM type polypeptide chain (SEQ ID No. 109 of the Sequence Listing) were prepared by PCR. Each of these sequences is a fusion of one the humanized versions of the variable region of the HFE7A light chain with the constant region of the human Ig light chain ( $\kappa$  chain).

7AL1P (SEQ ID No. 55) and 7ALCN (SEQ ID No. 64) had already been synthesized [Reference Example 2 (2) 2) above], and the following oligonucleotide primers were also synthesized for PCR:

5'- GGTGAGATTG TGCTCACCCA ATCTCCAGG -3'  
(LPD1P; SEQ ID No. 110);  
5'- CCTGGAGATT GGGTGAGCAC AATCTCACC -3'  
(LPD1N; SEQ ID No. 111);  
5'- CCATCTCTCG TCTGGAGCCG GAGGATTTG C -3'  
(LPD2P; SEQ ID No. 112);  
5'- GCAAAATCCT CCGGCTCCAG ACGAGAGATG G -3'  
(LPD2N; SEQ ID No. 113);  
5'- CAAGGCACCA AGCTGGAAAT CAAACGGACT G -3'  
(LPD3P; SEQ ID No. 114); and

5' - CAGTCCGTTT GATTTCCAGC TTGGTGCCCTT G -3'  
(LPD3N; SEQ ID No. 115).

2) Construction of plasmid pLPDHH75 (expression plasmid  
for humanized PDHH type HFE7A light chain)

LPDHH-DNA (light chain constant region fused with PDHH DNA), as defined in SEQ ID No. 106 of the Sequence Listing, and encoding the amino acid sequence of SEQ ID No. 107 of the Sequence Listing, was prepared by performing 3-stage PCR, inserted into a plasmid vector, and cloned into *E. coli*.

a) First stage PCR

The outline of the first stage PCR for the preparation of LPDHH-DNA is shown in Figure 31.

The LPD1-DNA fragment, encoding a secretion signal sequence and a portion of FRL<sub>1</sub>, but having an added HindIII restriction enzyme cleavage site at the 5'-end, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pHSGHH7 DNA, 200 ng;  
oligonucleotide primer 7AL1P, 80 pmol;  
oligonucleotide primer LPD1N, 80 pmol;  
dNTP cocktail, 20 µl;  
10x Pfu buffer, 20 µl;  
Pfu DNA polymerase (Stratagene), 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for

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1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The LPDHH1-DNA fragment, encoding a portion of FRL<sub>1</sub>, CDRL<sub>1</sub>, FRL<sub>2</sub>, CDRL<sub>2</sub> and a portion of the FRL<sub>3</sub> region, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pHSGHH7 DNA, 200 ng;  
oligonucleotide primer LPD1P, 80 pmol;  
oligonucleotide primer LPD2N, 80 pmol;  
dNTP cocktail, 20 µl;  
10x Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The LPDHH2-DNA fragment, encoding a portion of FRL<sub>3</sub>, CDRL<sub>3</sub> and FRL<sub>4</sub>, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pHSGHH7 DNA, 200 ng;  
oligonucleotide primer LPD2P, 80 pmol;  
oligonucleotide primer LPD3N, 80 pmol;  
dNTP cocktail, 20 µl;  
10x Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and

redistilled water to a final volume of 200  $\mu$ l.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The LPDC-DNA fragment, encoding a portion of FRL<sub>4</sub> and the HFE7A light chain constant region, but having an EcoRI restriction enzyme cleavage site added at the 3'-end, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pHSGHH7 DNA, 200 ng;  
oligonucleotide primer LPD3P, 80 pmol;  
oligonucleotide primer 7ALCN, 80 pmol;  
dNTP cocktail, 20  $\mu$ l;  
10x Pfu buffer, 20  $\mu$ l;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200  $\mu$ l.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After PCR, the amplified DNA fragments were subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1  $\mu$ g/ml of ethidium bromide, and the DNA bands thus detected, under UV light, were cut out with a razor blade and

eluted from the gel using a Centrifiruter and a Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 x g, followed by ethanol precipitation, and was finally dissolved in 50 µl of distilled water.

b) Second stage PCR

The outline of the second stage PCR for the production of LPDHH-DNA is shown in Figure 32.

LPDHH1.2-DNA, in which the above described LPD1-DNA, LPDHH1-DNA and LPDHH2-DNA fragments are fused, was prepared as follows.

Composition of the PCR reaction solution:

LPD1-DNA solution (from the first stage PCR), 10 µl;  
LPDHH1-DNA solution (from the first stage PCR), 10 µl;  
LPDHH2-DNA solution (from the first stage PCR), 10 µl;  
oligonucleotide primer 7AL1P, 80 pmol;  
oligonucleotide primer LPD3N, 80 pmol;  
dNTP cocktail, 20 µl;  
10x Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After PCR, the amplified LPDHH1.2 fragment was subjected first to phenol extraction and then to ethanol precipitation, and then separated

by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide, and the fusion DNA band thus detected, under UV light, was cut out with a razor blade and eluted from the gel using a Centrifiruter and a Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 x g, followed by ethanol precipitation, and was finally dissolved in 50 µl of distilled water.

c) Third stage PCR

The outline of the third stage PCR for the production of LPDHH-DNA is shown in Figure 33.

The LPDHH-DNA fragment, in which the above described LPDHH1.2-DNA and LPDC-DNA fragments were fused, was prepared as follows.

Composition of the PCR reaction solution:

LPDHH1.2-DNA solution (from second stage PCR), 10 µl;  
LPDC-DNA solution (from first stage PCR), 10 µl;  
oligonucleotide primer 7AL1P, 80 pmol;  
oligonucleotide primer 7ALCN, 80 pmol;  
dNTP cocktail, 20 µl;  
10x Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After PCR, the amplified LPDHH-DNA fragment was subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide, and the DNA band thus detected, under UV light, was cut out with a razor blade and eluted from the gel using a Centrifiruter and a Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 × g, followed by ethanol precipitation, and was finally dissolved in 50 µl of distilled water.

Construction of an expression plasmid carrying the LPDHH-DNA fragment is outlined in Figure 34.

The LPDHH-DNA fragment, obtained above, was further purified by phenol extraction followed by ethanol precipitation, and then digested with the restriction enzymes HindIII and EcoRI.

One µg of the cloning plasmid pHSG399 DNA was digested with the restriction enzymes HindIII and EcoRI, and then dephosphorylated with CIP. The resulting dephosphorylated pHSG399 DNA was then ligated with the LPDHH-DNA fragment, which had previously also been digested with HindIII and EcoRI, using a DNA Ligation Kit Version 2.0 (Takara Shuzo, Co. Ltd.). *E. coli* JM109 was then transformed with the ligation mix and plated onto LB agar medium containing final concentrations of 1 mM IPTG, 0.1% w/v X-Gal and 50 µg/ml chloramphenicol. Any white transformants obtained were cultured in 2 ml liquid LB medium containing 50 µg/ml chloramphenicol at 37°C overnight, and plasmid DNA was extracted from the resulting culture by the alkaline-SDS method. The extracted plasmid DNA was digested with HindIII and EcoRI, and a clone carrying the LPDHH-DNA fragment was then selected by 1% w/v agarose gel electrophoresis.

Accordingly, plasmid pHSHH5, carrying a fusion insert comprising the variable region of the humanized LPDHH DNA and DNA encoding the constant region of human immunoglobulin κ chain, was isolated. The transformant *E. coli* pHSHH5 SANK 70398, harboring plasmid pHSHH5, was deposited with the Kogyo Gijutsuin Seimei-Kogaku Kogyo Gijutsu Kenkyujo on February 26, 1998, in accordance with the Budapest Treaty on the Deposit of Microorganisms, and was accorded the accession number FERM BP-6274.

The expression vector plasmid pLPDHH75 carrying the DNA fragment of SEQ ID No. 106 of the Sequence Listing, encoding the humanized PDHH type HFE7A light chain polypeptide of SEQ ID No. 107 of the Sequence Listing, was then prepared using the plasmid pHSHH5.

One μg of pEE.12.1 DNA (Lonza), an expression vector for mammalian cells, was digested with the restriction enzymes HindIII and EcoRI, and then dephosphorylated using CIP. The resulting digested, dephosphorylated plasmid DNA (100 ng) was ligated with 10 μg of the pHSHH5 DNA fragment which had also been digested with Hind III and EcoRI, using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). The ligation mix was then used to transform *E. coli* JM109 (as described above), which was then plated on LB agar plates containing 50 μg/ml ampicillin.

The transformants obtained by this method were cultured in 2 ml of liquid LB medium containing 50 μg/ml ampicillin at 37°C overnight, and plasmid DNA was subsequently extracted from the resulting culture by the alkaline-SDS method.

The extracted plasmid DNA was digested with HindIII and EcoRI, and subjected to 1% w/v agarose gel electrophoresis to confirm the presence or absence of the insert of interest. This enabled the

isolation of the plasmid pLPDHH75, which contains a fusion fragment having the variable region of the humanized PDHH type HFE7A light chain together with DNA encoding the constant region of the human immunoglobulin κ chain. The fusion fragment was found to be located downstream of the cytomegalovirus (CMV) promoter in the correct orientation.

3) Construction of plasmid pLPDHM32 (expression plasmid for humanized PDHM type HFE7A light chain)

The LPDHM-DNA fragment (SEQ ID No. 108 of the Sequence Listing, encoding the amino acid sequence of SEQ ID No. 109 thereof) was produced by performing 3-stage PCR, the fragment then being inserted into a plasmid vector and cloned into *E. coli*.

a) First stage PCR

The outline of the first stage PCR for the preparation of LPDHM-DNA is shown in Figure 35.

The LPD1-DNA fragment, encoding a secretion signal sequence and a portion of FRL<sub>1</sub> having a HindIII restriction enzyme cleavage site added at the 5'-end, and the LPDC-DNA fragment, encoding a portion of FRL<sub>4</sub> and the constant region having an EcoRI restriction enzyme cleavage site added at the 3'-end, were those obtained in the preparation of the LPDHH-DNA fragment [see "2) Construction of plasmid pLPDHH75 (expression plasmid for humanized PDHH type HFE7A light chain)" and "a) First Stage PCR"].

The LPDHM1-DNA fragment, encoding a portion of CDRL<sub>1</sub>, FRL<sub>2</sub>, CDRL<sub>2</sub>, FRL<sub>3</sub>, CDRL<sub>3</sub> and FRL<sub>4</sub>, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pHSGHM17 DNA, 200 ng;  
oligonucleotide primer LPD1P, 80 pmol;  
oligonucleotide primer LPD3N, 80 pmol;  
dNTP cocktail, 20 µl;  
10x Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After PCR, the amplified LPD1, LPDHM1 and LPDC-DNA fragments were subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide, and the fusion DNA bands thus detected, under UV light, were cut out with a razor blade and eluted from the gel using a Centrifuter and a Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 x g, followed by ethanol precipitation, and was finally dissolved in 50 µl of distilled water.

b) Second stage PCR

The outline of the second stage PCR for the production of PDHM-DNA is shown in Figure 36.

LPDHM1.2-DNA, in which the above LPD1-DNA and LPDHM1-DNA fragments were fused, was prepared as follows.

Composition of the PCR reaction solution:

LPD1-DNA solution (from the first stage PCR), 10 µl;  
LPDHM1-DNA solution (from the first stage PCR), 10 µl;  
oligonucleotide primer 7AL1P, 80 pmol;  
oligonucleotide primer LPD3N, 80 pmol;  
dNTP cocktail, 20 µl;  
10x Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After PCR, the amplified LPDHM1.2-DNA fragment was subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide, and the DNA band thus detected, under UV light, was cut out with a razor blade and eluted from the gel using a Centrifiruter and a Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 x g, followed by ethanol precipitation, and was finally dissolved in 50 µl of distilled water.

c) Third stage PCR

The outline of the third stage PCR for the preparation of LPDHM-DNA is shown in Figure 37.

The LPDHM-DNA fragment, comprising a fusion of the LPDHM1.2-DNA and LPDC-DNA fragments above, was prepared as follows.

Composition of the PCR reaction solution:

LPDHM1.2-DNA solution (from the second stage PCR), 10 µl;  
LPDC-DNA solution (from the first stage PCR), 10 µl;  
oligonucleotide primer 7AL1P, 80 pmol;  
oligonucleotide primer 7ALCN, 80 pmol;  
dNTP cocktail, 20 µl;  
10x Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After PCR, the amplified LPDHM-DNA fragment was subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide, and the DNA band thus detected, under UV light, was cut out with a razor blade and eluted from the gel using a Centrifiruter and a Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 × g, followed by ethanol precipitation, and was finally dissolved in 50 µl of distilled water.

The construction of a plasmid carrying the LPDHM-DNA fragment is outlined in Figure 38.

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The LPDHM-DNA obtained above was further purified by phenol extraction followed by ethanol precipitation, and then digested with the restriction enzymes HindIII and EcoRI.

One  $\mu$ g of the cloning plasmid pHSG399 DNA was digested with the restriction enzymes HindIII and EcoRI, and then dephosphorylated with CIP. The resulting dephosphorylated pHSG399 DNA and was then ligated with the LPDHM-DNA fragment, which had previously also been digested with HindIII and EcoRI, using a DNA Ligation Kit Version 2.0 (Takara Shuzo, Co. Ltd.). *E. coli* JM109 was then transformed with the ligation mix and plated onto LB agar medium containing final concentrations of 1 mM IPTG, 0.1% w/v X-Gal and 50  $\mu$ g/ml chloramphenicol. Any white transformants obtained were cultured in 2 ml liquid LB medium containing 50  $\mu$ g/ml chloramphenicol at 37°C overnight, and plasmid DNA was extracted from the resulting culture by the alkaline-SDS method. The extracted plasmid DNA was digested with HindIII and EcoRI, and a clone carrying the LPDHM-DNA fragment was then selected by 1% w/v agarose gel electrophoresis.

As a result of the above procedure, plasmid pHSHM2 carrying a fusion insert comprising the variable region of the humanized PDMM type HFE7A light chain and DNA encoding the constant region of the human Ig  $\kappa$  chain, was obtained. The transformant *E. coli* pHSHM2 SANK 70198, harboring plasmid pHSHM2, was deposited with the Kogyo Gijutsuin Seimei-Kogaku Kogyo Gijutsu Kenkyujo on February 26, 1998, in accordance with the Budapest Treaty on the Deposit of Microorganisms, and was accorded the accession number FERM BP-6272.

The expression vector plasmid pLPDHM32 was constructed, carrying the DNA of SEQ ID No. 108 of the Sequence Listing encoding the humanized PDHM type HFE7A light chain polypeptide of SEQ ID No. 109, using plasmid pHSHM2 obtained above.

One  $\mu$ g of pEE.12.1 DNA (Lonza), an expression vector for mammalian cells, was digested with the restriction enzymes Hind III and EcoRI, and then dephosphorylated using CIP. The resulting digested, dephosphorylated plasmid DNA (100 ng) was ligated with 10  $\mu$ g of the pHSHM2 DNA fragment which had also been digested with Hind III and EcoRI, using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). The ligation mix was then used to transform *E. coli* JM109 (as described above), which was then plated on LB agar plates containing 50  $\mu$ g/ml ampicillin.

The transformants obtained by this method were cultured in 2 ml of liquid LB medium containing 50  $\mu$ g/ml ampicillin at 37°C overnight, and plasmid DNA was subsequently extracted from the resulting culture by the alkaline-SDS method.

The extracted plasmid DNA was digested with HindIII and EcoRI, and subjected to 1% w/v agarose gel electrophoresis to confirm the presence or absence of the insert of interest. This enabled the isolation of the plasmid pLPDHH32, which contains a fusion fragment having the variable region of the humanized PDHM type HFE7A light chain together with DNA encoding the constant region of the human immunoglobulin  $\kappa$  chain. The fusion fragment was found to be located downstream of the cytomegalovirus (CMV) promoter in the correct orientation.

#### (4) Verification of nucleotide sequences

To verify that the DNA inserts of plasmids pLPDHH75 and pLPDHH32 have the desired nucleotide sequences, the DNA inserts were analyzed to determine their nucleotide sequences. The oligonucleotide primers used for nucleotide sequencing were SP1 (SEQ ID No. 68), SP2 (SEQ ID No. 69),

SP3 (SEQ ID No. 70), SP4 (SEQ ID No. 71), SP5 (SEQ ID No. 72) and SP6 (SEQ ID No. 73).

The positions to which each primer binds are shown in Figure 19. The determination of the nucleotide sequence was performed by the dideoxynucleotide chain termination method using, as the templates, the plasmid containing the sequence to be confirmed, the plasmid having been purified by the alkaline-SDS method and the cesium chloride method. As expected pLPDHH75 was confirmed to have the nucleotide sequence of SEQ ID No. 106 of the Sequence Listing, encoding the polypeptide of SEQ ID No. 107, and that pLPDHM32 had the nucleotide sequence of SEQ ID No. 108 of the Sequence Listing, encoding the polypeptide of SEQ ID No. 109.

REFERENCE EXAMPLE 22

Preparation of DNA Encoding Humanized Heavy Chain

- (1) Construction of vector for the heavy chain of humanized version of HFE7A antibody

In further humanizing the amino acid sequence of SEQ ID No. 75 of the Sequence Listing (the humanized heavy chain of the mouse anti-human Fas antibody HFE7A), the 44th amino acid (arginine) and the 76th amino acid (alanine) in the amino acid sequence of SEQ ID No. 75 were replaced with glycine and threonine, respectively, these residues being conserved in the human heavy chain. The resulting sequence was designated as the "HV type."

Expression plasmids carrying the HV type humanized heavy chain amino acid sequences of the anti-human Fas antibody HFE7A were constructed as follows.

(1) Synthesis of primers for preparing the variable region of the humanized heavy chain

The synthesis of DNA (SEQ ID No. 116 of the Sequence Listing) encoding the humanized anti-Fas antibody HFE7A heavy chain (SEQ ID No. 117 of the Sequence Listing) was performed by using a combination of PCR steps.

In addition to 7AH1P (SEQ ID No: 76 above), the following 3 primers were synthesized for PCR:

5'- CAGGCCCTG GACAGGGCCT TGAGTGGATG -3'  
(HPD1P; SEQ ID No. 118);  
5'- CATCCACTCA AGGCCCTGTC CAGGGGCCTG -3'  
(HPD1N; SEQ ID No. 119); and  
5'- GCTGAGCTCC ATGTAGGCTG TGCTAGTGGGA TGTGTCTAC -3'  
(HPD2N; SEQ ID No. 120).

2) Construction of plasmid pgHPDHV3

The HPD1.2-DNA fragment, encoding amino acid No's -19 - +84 of SEQ ID No. 117 of the Sequence Listing, was prepared by performing 2-stage PCR, inserted into a plasmid and then cloned into *E. coli*.

a) First stage PCR

The outline of the first stage PCR for the preparation of HPD1.2-DNA is shown in Figure 39.

The HPD1-DNA fragment, encoding a secretion signal sequence and FRH<sub>1</sub>, CDRH<sub>1</sub> and a portion of FRH<sub>2</sub> with an added HindIII restriction enzyme cleavage site added at the 5'-end, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pgHSL7A62 DNA, 200 ng;  
oligonucleotide primer 7AH1P, 80 pmol;  
oligonucleotide primer HPD1N, 80 pmol;  
dNTP cocktail, 20  $\mu$ l;  
10x Pfu buffer, 20  $\mu$ l;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200  $\mu$ l.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The HPD2-DNA fragment, encoding a portion of FRH<sub>2</sub>, CDRH<sub>3</sub>, and a portion of FRH<sub>3</sub>, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pgHSL7A62 DNA, 200 ng;  
oligonucleotide primer HPD1P, 80 pmol;  
oligonucleotide primer HPD2N, 80 pmol;  
dNTP cocktail, 20  $\mu$ l;  
10x Pfu buffer, 20  $\mu$ l;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200  $\mu$ l.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After PCR, the amplified HPD1 and HPD2 DNA fragments were subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide, and the DNA bands thus detected, under UV light, were cut out with a razor blade and eluted from the gel using a Centrifiruter and a Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 × g, followed by ethanol precipitation, and was finally dissolved in 50 µl of distilled water.

b) Second stage PCR

The outline of the second stage PCR for the preparation of HPD1.2-DNA is shown in Figure 40.

The HPD1.2-DNA fragment, in which above described HPD1-DNA and HPD2-DNA fragments are fused, was prepared as follows.

Composition of the PCR reaction solution:

HPD1-DNA solution (from the first stage PCR), 10 µl;  
HPD2-DNA solution (from the first stage PCR), 10 µl;  
oligonucleotide primer 7AH1P, 80 pmol;  
oligonucleotide primer HPD2N, 80 pmol;  
dNTP cocktail, 20 µl;  
10x Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for

1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After PCR, the amplified HPD1.2 DNA fragment was subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide, and the DNA band thus detected, under UV light, was cut out with a razor blade and eluted from the gel using a Centrifuter and a Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 x g, followed by ethanol precipitation, and was finally dissolved in 50 µl of distilled water.

The construction of a plasmid carrying HPD1.2-DNA fragment is outlined in Figure 41.

The HPD1.2-DNA fragment obtained above was further purified by phenol extraction followed by ethanol precipitation, and then digested with the restriction enzymes HindIII and SacI.

Next, 10 µg of the plasmid pgHSL7A62 DNA was digested with the restriction enzymes HindIII and SacI and dephosphorylated with CIP. The resulting dephosphorylated pgHSL7A62 DNA (100 ng), was ligated with 10 µg of HPD1.2-DNA, which had previously been digested with HindIII and SacI, using a DNA Ligation Kit Version 2.0 (Takara Shuzo, Co. Ltd.) and the ligation mix was cloned into *E. coli* JM109. Any resulting transformants were cultured in 2 ml liquid LB medium, containing 50 µg/ml chloramphenicol at 37°C overnight, and plasmid DNA was extracted from the culture by the alkaline-SDS method.

The extracted plasmid was then digested with HindIII and SacI, in order to confirm the presence or absence of the insert of interest by 1% w/v agarose gel electrophoresis. Thus, the plasmid pgHPDHV3, carrying a fusion insert comprising the DNA fragment encoding the variable region of the humanized HV type HFE7A heavy chain and a genomic DNA fragment encoding the constant region of human IgG1 heavy chain, was obtained. The transformant *E. coli* pgHPDHV3 SANK 70298, harboring plasmid pgHPDHV3, was deposited with the Kogyo Gijutsuin Seimei-Kogaku Kogyo Gijutsu Kenkyujo on February 26, 1998, in accordance with the Budapest Treaty on the Deposit of Microorganisms, and was accorded the accession number FERM BP-6273.

Ten micrograms of the thus obtained plasmid pgHPDHV3 DNA was digested with the restriction enzymes HindIII and EcoRI. In parallel, 1 µg of the mammalian expression plasmid pEE.6.1 DNA was digested with HindIII and EcoRI, and then dephosphorylated with CIP. The resulting dephosphorylated pEE.6.1 DNA (100 ng) was ligated with 10 µg of digested pgHPDHV3 DNA using a ligation kit Version 2.0 (Takara Shuzo, Co. Ltd.), and cloned into *E. coli* JM109. Any resulting transformants were cultured in 2 ml liquid LB medium containing 50 µg/ml ampicillin at 37°C overnight, and plasmid DNA was extracted from the culture by the alkaline-SDS method. The plasmid was digested with HindIII and EcoRI, to confirm the presence or absence of the insert of interest by 1% w/v agarose gel electrophoresis. Thus, the plasmid pEgPDHV3-21, containing a fusion insert comprising the DNA fragment encoding the variable region of the humanized HV type HFE7A heavy chain and a genomic DNA fragment encoding the constant region of human IgG1 heavy chain downstream of CMV promoter, and in the correct orientation, was obtained.

(3) Verification of nucleotide sequence

To verify that the DNA insert of the plasmid pEgPDHV3-21 had the

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desired nucleotide sequence, the DNA insert was analyzed to determine the nucleotide sequence. For this sequencing, the primers PEEF (SEQ ID No. 104), HPD1P (SEQ ID No. 1118), HPD1N (SEQ ID No. 119) and HPD2N (SEQ ID No. 120) were used.

The positions, to which the primers bind, are shown in Figure 42. Determination of nucleotide sequences was performed by the dideoxynucleotide chain termination method using, as templates, the plasmids, purified by the alkaline-SDS method and the cesium chloride method, containing the sequences for confirmation.

As expected, it was verified that pEgPDHV3-21 had the nucleotide sequence of SEQ ID No. 116 of the Sequence Listing, encoding the polypeptide of SEQ ID No. 117.

#### REFERENCE EXAMPLE 23

##### Construction of High-Level Expression Vectors Optimized for COS-1 Cells

High-level expression vectors, optimized for COS-1 cells, were constructed, using the above described vectors p7AL-HH, p7AL-HM, p7AL-MM, pLPDHH75, pLPDHM32, pEg7AH-H and pEgPDHV3-21.

###### (1) High-level expression vectors for humanized light chains

The construction of high-level expression vectors for the humanized light chains is outlined in Figure 43.

- 1) Synthesis of primers for preparing the SR  $\alpha$  promoter DNA fragment

The SR  $\alpha$  promoter DNA fragment, for insertion into the expression vectors for humanized light chains, was synthesized using PCR.

The following 2 oligonucleotide primers were synthesized for PCR:

5' - TGCACGCGTG GCTGTGGAAT GTGTGTCAGT TAG -3'

(MLUA: SEQ ID No. 121); and

5' - TCCGAAGCTT TTAGAGCAGA AGTAACACTT C -3'

(HINDB: SEQ ID No. 122).

## 2) Construction of plasmids

After synthesis, the SR  $\alpha$  promoter DNA fragment was inserted into the vectors p7AL-HH, p7AL-HM, p7AL-MM, pLPDHH75 or pLPDHM32 and then cloned into *E. coli*.

An LSR  $\alpha$ -DNA fragment, comprising the SR  $\alpha$  promoter with a MluI restriction enzyme cleavage site added at the 5'-end and a HindIII restriction enzyme cleavage site added at the 3'-end, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pME18S DNA, 200 ng;

oligonucleotide primer MLUA, 80 pmol;

oligonucleotide primer HINDB, 80 pmol;

dNTP cocktail, 20  $\mu$ l;

10x Pfu buffer, 20  $\mu$ l;

Pfu DNA polymerase, 10 units; and

redistilled water to a final volume of 200  $\mu$ l.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30

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times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After PCR, the amplified LSR α-DNA fragment was subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide, and the DNA band thus detected, under UV light, was cut out with a razor blade and eluted from the gel using a Centrifiruter and a Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500 × g, followed by ethanol precipitation, and was finally dissolved in 50 µl of distilled water.

One µg of plasmid p7AL-HH, p7AL-HM, p7AL-MM, pLPDHH75 or pLPDHM32 DNA was digested with the restriction enzymes MluI and HindIII, and then dephosphorylated with CIP. The resulting dephosphorylated plasmid DNA (100 ng) was ligated with 10 µl of the solution containing the LSR α-DNA fragment, which had previously been digested with MluI and HindIII, using a DNA Ligation Kit Version 2.0 (Takara Shuzo, Co. Ltd.). *E. coli* JM109 was then transformed with the ligation mix and plated onto LB agar medium containing 50 µg/ml ampicillin. The transformants obtained were cultured in 2 ml liquid LB medium containing 50 µg/ml ampicillin at 37°C overnight, and plasmid DNA was extracted from the resulting culture by the alkaline-SDS method. The extracted plasmid DNA was digested with MluI and HindIII, and a clone carrying LSR α-DNA fragment was selected by 1% w/v agarose gel electrophoresis.

Following the above procedure, the high-level expression vector plasmids pSRHH (HH type), pSRHM (HM type), pSRMM (MM type), pSRPDHH (PDHH type) and pSRPDHM (PDHM type), were obtained.

(2) High-level expression vectors for humanized heavy chains

The construction of high-level expression vectors for humanized heavy chains is outlined in Figure 44.

1) Synthesis of primers for preparing SR  $\alpha$  promoter DNA fragment

The SR  $\alpha$  promoter DNA fragment, for insertion into the expression vectors for humanized heavy chains, was synthesized using PCR.

In addition to HINDB (SEQ ID No 122), the following oligonucleotide primer was synthesized for PCR:

5' - AAAGCGGCCG CTGCTAGCTT GGCTGTGGAA TGTGTG - 3'  
(NOTA: SEQ ID NO. 123).

2) Construction of plasmids

The SR  $\alpha$  promoter DNA fragment was synthesized using PCR, inserted into the above described vector, pEg7AH-H or pEgPDHV3-21, and then cloned into *E. coli*.

The HSR  $\alpha$ -DNA fragment, comprising the SR  $\alpha$  promoter with a NotI restriction enzyme cleavage site added at the 5'-end and a HindIII restriction enzyme cleavage site added at the 3'-end, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pME18S DNA, 200 ng;  
oligonucleotide primer NOTA, 80 pmol;

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oligonucleotide primer HINDB, 80 pmol;  
dNTP cocktail, 20  $\mu$ l;  
10x Pfu buffer, 20  $\mu$ l;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200  $\mu$ l.

The PCR reaction was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After PCR, the amplified HSR  $\alpha$ -DNA fragment was subjected first to phenol extraction and then to ethanol precipitation, and then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1  $\mu$ g/ml of ethidium bromide, and the DNA band thus detected, under UV light, was cut out with a razor blade and eluted from the gel using a Centrifuter and a Centricon-10, as described above. The eluted DNA was concentrated by centrifugation at 7,500  $\times g$ , followed by ethanol precipitation, and was finally dissolved in 50  $\mu$ l of distilled water.

One  $\mu$ g of plasmid pEg7AH-H or pEgPDHV3-21 DNA was then digested with the restriction enzymes NotI and HindIII, and then dephosphorylated with CIP. The resulting dephosphorylated plasmid DNA (100 ng) was ligated with 10  $\mu$ l of the solution containing the HSR  $\alpha$ -DNA fragment, which had previously also been digested with NotI and HindIII, using a DNA Ligation Kit Version 2.0 (Takara Shuzo, Co. Ltd.). *E. coli* JM109 was transformed with the ligation mix and plated onto LB agar medium containing final concentrations of 1 mM IPTG, 0.1% w/v X-Gal and 50  $\mu$ g/ml ampicillin. The white transformants obtained were cultured in 2 ml liquid LB medium containing 50  $\mu$ g/ml ampicillin at 37°C overnight,

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and plasmid DNA was extracted from the resulting culture by the alkaline-SDS method. The extracted plasmid DNA was digested with NotI and HindIII, and a clone carrying then HSR  $\alpha$ -DNA fragment was selected by 1% w/v agarose gel electrophoresis.

By following the above procedure, the high-level expression vector plasmids pSRg7AH and pSRgPDH (HV type) were obtained.

REFERENCE EXAMPLE 24

Expression in COS-1 Cells

Transfection of COS-1 cells with the high-level expression plasmids, for each of the humanized HFE7A heavy chains and for each of the humanized HFE7A light chains obtained above, was conducted by electroporation in a manner similar to that described in Reference Example 16.

COS-1 cells were grown to semi-confluence in a culture flask (culture area: 225 cm<sup>2</sup>), containing  $\alpha$ (+)-MEM supplemented with 10% v/v FCS. Next, the medium was discarded and the COS-1 cells were detached from the flask by treatment with 3 ml of trypsin-EDTA solution (Sigma Chemicals Co.) at 37°C for 3 minutes. The detached cells were then harvested by centrifugation at 800 r.p.m. for 2 minutes and then washed twice with PBS(-) buffer. The washed COS-1 cells were then adjusted to 1  $\times$  10<sup>8</sup> cells/ml with PBS(-) buffer to produce a COS-1 cell suspension.

In parallel, 4  $\mu$ g of humanized HFE7A heavy chain expression plasmid DNA and 4  $\mu$ g of humanized HFE7A light chain expression plasmid DNA, prepared by the alkaline-SDS method and cesium chloride density gradient centrifugation, were mixed and then precipitated with ethanol, before being suspended in 20  $\mu$ l of PBS(-) buffer, the whole operation

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being performed in the same tube. The whole of the resulting plasmid suspension (20 µl) was mixed with 20 µl of the previously prepared COS-1 cell suspension ( $2 \times 10^6$  cells) and the mixture was transferred to a FCT-13 (Shimadzu Seisakusyo, K. K.) chamber having electrodes set 2 mm apart. This chamber was then loaded into gene transfection apparatus GTE-1 (Shimadzu Seisakusyo, K. K.) and two pulses, each of 600 V, 50 µF, were applied with a one second interval to transform the COS-1 cells with the plasmid DNA of interest.

After electroporation, the cell-DNA mixture in the chamber was suspended in 5 ml of α(+)MEM, supplemented with 10% v/v FCS, in a culture flask (culture area 25 cm<sup>2</sup>; Sumitomo Bakelite) and incubated under 5% v/v CO<sub>2</sub> at 37°C for 72 hours. After this time, the culture supernatant was taken and analyzed for the expression products.

By following the above method, COS-1 cells transfected with each of the following plasmid combinations were obtained:

- (A) no plasmid DNA;
- (B) cotransfection of pSRgPDH and pSRPDHH;
- (C) cotransfection of pSRgPDH and pSRPDHM;
- (D) cotransfection of pSRg7AH and pSRHH;
- (E) cotransfection of pSRg7AH and pSRHM; and
- (F) cotransfection of pSRg7AH and pSRMM.

REFERENCE EXAMPLE 25

Assay for Fas-Binding Activity

The assay for Fas-binding activity in the cell culture supernatant fluids prepared in Reference Example 24 was performed by ELISA as follows.

Culture supernatant from COS-1 cells expressing the human Fas fusion protein, as obtained in Reference Example 1 above, diluted 5-fold with adsorption buffer, was dispensed into wells of a 96-well plate (MaxiSorb; Nunc) at 50 µl per well and the plate was incubated at 4°C overnight to allow adsorption of the human Fas fusion protein to the surface of the wells. Next, each of the wells was washed 4 times with 350 µl of PBS-T. After washing, PBS-T containing 5% v/v BSA (bovine serum albumin; Wako Pure Chemical Industries, Ltd.) was added to the wells at 50 µl per well and the plate was incubated at 37°C for 1 hour to block the remainder of the surface of each well. The wells were then again washed four times with PBS-T.

The culture supernatants obtained in Reference Example 16 were adjusted to have a final concentration of the product of interest of 100 ng/ml in α(+)MEM containing 10% v/v FCS. Concentrations were estimated by the method described in Reference Example 17. Each of the resulting 100 ng/ml solutions was then used to produce serial dilutions by serial 2-fold dilution with α(+)MEM containing 10% v/v FCS. Next, 50 µl of each of the resulting serial dilutions of each expression product was added to a well prepared as above, and the plate was incubated at 37°C for 2 hours to allow reaction.

After this time, the wells were again washed four times with PBS-T, and then 50 µl of alkaline phosphatase-labeled goat anti-human IgG Fc specific polyclonal antibody (Caltag Lab.), diluted 10,000-fold with PBS-T, were dispensed into each well and reaction was allowed to proceed at 37°C for 2 hours.

HFE7A purified from mouse hybridoma HFE7A was used as a control (IgG1), and was detected using alkaline phosphatase-labeled goat anti-mouse IgG + IgA + IgM (Gibco BRL), diluted 5,000-fold with PBS-T, in

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place of the alkaline phosphatase-labeled goat anti-human IgG Fc specific polyclonal antibody.

The wells were again washed four times with PBS-T, and then 50 µl of substrate solution [1 mg/ml p-nitrophenyl phosphate in 10% v/v diethanol amine (pH 9.8)] was dispensed into each well and the plate was incubated at 37°C for 0.5 to 1 hour. Binding activity of the expression product contained in each culture supernatant fluid with the human Fas fusion protein was evaluated by reading the absorbance of each well at 405 nm.

As expected, binding activity for the human Fas fusion protein was demonstrated for the supernatants of categories (B), (C), (D), (E) and (F) above of Reference Example 24, and is shown in Figure 45.

#### REFERENCE EXAMPLE 26

##### Competitive Inhibition of Fas-Binding Activity of HFE7A

The humanized anti-Fas antibodies obtained in Reference Example 24 should inhibit the binding of HFE7A to Fas, as the antibodies of this Example were derived from HFE7A. Therefore, the ability of the expression products obtained in Reference Example 24 to competitively inhibit the binding of HFE7A to the human Fas fusion protein was measured.

The COS-1 cell culture supernatant containing the human Fas fusion protein, as obtained in Reference Example 1, was diluted 5-fold with adsorption buffer, and dispensed into the wells of a 96-well plate for luminescence detection (Luminescent Solid Assay Plate, high binding property; Costar) at 50 µl per well. The plate was then incubated at

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4°C overnight to allow adsorption of the human Fas fusion protein to the surface of the wells.

After this time, each well was washed 4 times with 350 µl of PBS-T, and then 100 µl PBS-T containing 5% v/v BSA was added to each well and the plate was incubated at 37°C for 1 hour to block the remainder of the surface of each well. The wells were then again washed four times with PBS-T.

The culture supernatants obtained in Reference Example 24 were adjusted to final concentrations of antibody of 1 µg/ml in α(+)MEM containing 10% v/v FCS by the method of Reference Example 17. Each of the resulting solutions of the expression products was used to produce serial dilutions by serial 2-fold dilution with α(+)MEM containing 10% v/v FCS. AP-HFE7A was diluted to 50 ng/ml with α(+)MEM containing 10% v/v FCS, and 25 µl of the resulting solution was mixed with an equal volume of each of the prepared serial dilutions.

Each of the wells was again washed four times with PBS-T, and then 50 µl of each of the resulting antibody mixtures were added to individual wells, and the plate was allowed to stand at room temperature overnight. Subsequently, after washing each well with PBS-T again four times, 100 µl of CDP-star buffer (9.58 ml diethanol amine, 0.2 g magnesium chloride, 0.25 g sodium azide, pH8.5) was dispensed into each well and the plate was allowed to stand at room temperature for 10 minutes. After this time, the CDP-star buffer was discarded and CDP-star substrate [1.2 ml sapphire II (Tropix), 200 µl CDP-star (Tropix), q.s. to 12 ml with CDP-star buffer] was added at 50 µl per well, and the plate was then allowed to stand at room temperature for a further 40 minutes.

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Competitive inhibition of the expression products of Reference Example 24 of the binding of HFE7A to the human Fas fusion protein was evaluated by measuring the intensity of the luminescence with Luminoscan (Titertech).

As expected, it was verified that each of the expression products of supernatants (B), (C), (D), (E) and (F) obtained in Reference Example 24 above specifically inhibited the binding of HFE7A antibody to the human Fas fusion protein. The results are shown in Figure 46.

REFERENCE EXAMPLE 27

Apoptosis-Inducing Activity

The apoptosis-inducing activity of the expression products in the culture supernatant fluids obtained in Reference Example 24 was examined in a manner similar to that described in Reference Example 20.

WR19L12a cells were cultured in RPMI 1640 medium with 10% v/v FCS (Gibco BRL) at 37°C for 3 days under 5% v/v CO<sub>2</sub>, and 50 µl (1 × 10<sup>5</sup> cells) of the resulting culture were then dispensed into each well of a 96-well microplate (Sumitomo Bakelite). The culture supernatants obtained in Reference Example 24 were adjusted to a final concentration of antibody of 100 ng/ml in RPMI 1640 medium containing 10% v/v FCS. Concentrations were estimated by the method of Reference Example 17. Each of the adjusted solutions of the expression products was used to produce serial dilutions by serial 2-fold dilution with RPMI 1640 containing 10% v/v FCS. Each of the resulting dilutions of each expression product solution was added to individual wells, at 50 µl per well, and the plate was incubated at 37°C for 1 hour. After this time, the cells in each well were washed once with RPMI 1640 containing 10% v/v FCS and then the washed cells were suspended in 100 µl per well

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of 0.5 µg/ml goat anti-human IgG Fc specific polyclonal antibody (Kappel) in RPMI 1640 containing 10% v/v FCS.

The plate was allowed to stand at 37°C for 12 hours, and then 50 µl of 25 µM PMS (phenazine methosulfate; Sigma Chemical Co.), containing 1 mg/ml XTT [2,3-bis(2-methoxy-4-nitro-5-sulfophenyl)-2H-tetrazolium-5-carboxyanilide inner salt; Sigma Chemical Co.] to final concentrations of 333 µg/ml for XTT and 8.3 µM for PMS, were added to each well. The plate was then incubated for 3 hours at 37°C, and the absorbance at 450 nm of each well was measured, to calculate cell viability, using the reducing power of the mitochondria as the index.

The viability of the cells in each well was calculated according to the following formula:

$$\text{Viability (\%)} = 100 \times (a-b) / (c-b)$$

wherein "a" is the absorbance of a test well, "b" is the absorbance of a well with no cells, and "c" is the absorbance of a well with no antibody added.

As expected, each of the expression products of the culture supernatant fluids (B), (C), (D), (E) and (F) obtained in Reference Example 24 above were demonstrated to induce apoptosis in T cells of this lymphoma cell line expressing human Fas antigen (Figure 47).

### EXAMPLE 1

#### Designing an Eu type Humanized HFE7A Antibody

In the above Reference Examples, the human antibody 8E10'CL was chosen as an acceptor. In an alternative aspect of the present invention, the human Eu antibody was selected as an acceptor instead, in order to prepare the humanized antibody (hereinafter the humanized antibody, or a subunit thereof, as prepared in the Reference Examples, is referred to as "8E10 type", while the humanized HFE7A antibody, or subunit thereof, having Eu as the acceptor, is referred to as "Eu type").

##### (1) Molecular modeling of the variable regions of HFE7A

Molecular modeling of the variable regions of HFE7A was performed by the method generally known as homology modeling [c.f. Methods in Enzymology, 203, 121-153, (1991)].

The primary sequences of variable regions of human immunoglobulins registered in the Protein Data Bank (hereinafter referred to as the "PDB"; Chemistry Department, Building 555, Brookhaven National Laboratory, P.O. Box 5000, Upton, NY 11973-5000, USA), for which X-ray crystallography had been performed, were compared with the framework regions of HFE7A determined above. As a result, 1GGI and 2HFL were selected as having the highest homologies of the three-dimensional structures of the framework regions for the light and heavy chains, respectively. Three-dimensional structures of the framework regions were generated by combining the properties of 1GGI and 2HFL and by calculating the properties of the regions of HFE7A, as described below, thereby to obtain the "framework model".

Using the classification described by Chothia et al., the CDR's of HFE7A were classified as follows: CDRL<sub>2</sub>, CDRL<sub>3</sub> and CDRH<sub>1</sub> all belonged to canonical class 1, while CDRL<sub>1</sub>, CDRH<sub>2</sub> and CDRH<sub>3</sub> did not appear to belong to any specific canonical class. The CDR loops of CDRL<sub>2</sub>, CDRL<sub>3</sub> and CDRH<sub>1</sub> were ascribed the conformations inherent to their respective canonical classes, and then integrated into the framework model. CDRL<sub>1</sub> was assigned the conformation of cluster 15B, in accordance with the classification of Thornton et al. [c.f. J. Mol. Biol., 263, 800-815, (1996)]. For CDRH<sub>2</sub> and CDRH<sub>3</sub>, conformations of sequences with high homologies were selected from the PDB and then these were combined with the results of energy calculations. The conformations of the CDR loops with the highest probabilities were then taken and integrated into the framework model.

Finally, energy calculations were carried out to eliminate undesirable contact between inappropriate atoms, in terms of energy, in order to obtain a molecular model of HFE7A. The above procedure was performed using the commercially available common molecular modeling system, AbM (Oxford Molecular Limited, Inc.), although any other appropriate system could have been used.

The accuracy of the structure of the molecular model obtained was further evaluated using PROCHECK software [J. Appl. Cryst., (1993), 26, 283-291], and the degree of surface exposure of each residue was calculated to determine which surface atoms and groups interacted.

## (2) Selection of the acceptor

The subgroups of the light and heavy chains of HFE7A shared identities of 79% with the subgroup κIV and 79% with the subgroup I, respectively, by comparison with the consensus sequences of the respective subgroups of human antibodies. However, there was

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no human antibody having such a combination of these subgroups. Human antibody Eu was selected as an antibody in which the light chain and heavy chain were from the same antibody and which had the highest possible homology with HFE7A.

The antibody Eu has commonly been used to humanize antibodies (for the amino acid sequence of Eu, *c.f.* Kabat, E. A., *et al.*, *ibid.*). The light chain and the heavy chain of Eu belong to subgroup KI and subgroup I respectively.

(3) Selection of donor residues to be grafted onto the acceptor

The amino acid sequence of each of the light and heavy chains of HFE7A was aligned with that of the corresponding chain of the acceptor, and humanized sequences of the variable regions, as described in the following Examples, were designed in accordance with the general guidelines set out in a) to d) above.

Plasmids were constructed which could serve as recombinant vectors comprising DNA nucleotide sequences encoding Eu type, humanized, anti-Fas antibodies. Specifically, the vectors comprising DNA encoding 8E10 type humanized light chain and heavy chains, prepared in Reference Examples 14 and 15, were used as templates and modified so that the amino acid sequence in the FR was of the Eu type (Figures 48 and 49).

DNA encoding the constant region of the light chain, obtained by cloning, was ligated with DNA encoding the variable region. The constant region of the 8E10 type heavy chain was used without modification, as it was the same as that of the Eu type heavy chain.

EXAMPLE 2Construction of Expression Vector of Light Chain of  
Eu type Humanized HFE7A(1) Cloning of cDNA encoding a human light chain ( $\kappa$  chain subgroup type I)

Prior to preparation of the light chain of Eu type humanized HFE7A, cloning of cDNA of a human light chain  $\kappa$  chain subgroup, type I, was performed.

## 1) Synthesis of primer

Synthesis of a cDNA primer encoding the human light chain ( $\kappa$  chain subgroup type I) was carried out by PCR. For the PCR, the following oligonucleotide primer was synthesized::

5'-AAGCTTATGG ACATGAGGGT CCCCCGCTCTG CTCC-3'

(FHKI : SEQ ID No. 124 of the Sequence Listing), and used in combination with 7ALCN (SEQ ID No. 64 of the Sequence Listing).

## 2) Construction of a plasmid

An SpHE fragment encoding a full-length of human immunoglobulin light chain, having  $\kappa$  chain subgroup type I in the variable region, was prepared under the following conditions:

## Composition of the PCR reaction solution:

human spleen cDNA library (Life Technologies), 25 ng;

primer FHKI (10  $\mu$ M), 5  $\mu$ l;

primer 7ALCN (10  $\mu$ M), 5  $\mu$ l;

2.5 mM dNTP cocktail, 5  $\mu$ l;

25 mM Tris-HCl buffer (pH 8.2), 5  $\mu$ l;

1 M potassium chloride, 2.5  $\mu$ l;

25 mM magnesium chloride, 5  $\mu$ l;

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Taq DNA polymerase, 1 unit; and  
Redistilled water to a total volume of 50  $\mu$ l.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The thus prepared SpHE-DNA fragment was inserted into plasmid pCR3.1 using a eukaryote TA Cloning Kit (Invitrogen), following the manufacturer's protocol, and introduced into competent *E. coli* TOP10F' contained in the kit. Plasmid pKISp35 was thereby obtained, carrying the SpHE-DNA fragment, which is a cDNA of a human immunoglobulin light chain having  $\kappa$  chain subgroup type I in the variable region.

### 3) Nucleotide Sequence Analysis

The nucleotide sequences of the SpHE-DNA fragment carried by the plasmids pKISp35, obtained in (2) above, were determined by the dideoxy method [c.f. Sanger, F. S., et al., (1977), Proc. Natl. Acad. Sci. USA, 74: 5463-5467] using a gene sequence analyzer (Model 310 Genetic Analyzer; Perkin Elmer Japan). As a result, the nucleotide sequence of SEQ ID No. 125 of the Sequence Listing was obtained. The sequence corresponded to the nucleotide sequence of cDNA encoding a human Ig light chain having  $\kappa$  chain subgroup type I in the variable region.

### (2) Construction of expression plasmid vectors for Eu type humanized HFE7A light chain

cDNA's were prepared encoding three types of Eu-type humanized light chain amino acid sequences, modified so that FR's of the humanized HFE7A light chain resembled those of an Eu type

molecule (subgroup type I) as shown in Figure 48 (hereinafter referred to as "LEU1 type", "LEU2 type" and "LEU3 type"). The cDNA's were prepared according to the following method, and each of them was inserted into a plasmid.

(1) **Synthesis of primers**

PCR was used to construct the following DNA sequences, each of which comprised the variable region of the light chain of Eu type humanized HFE7A and the constant region of the human immunoglobulin light chain ( $\kappa$  chain):

DNA (SEQ ID No. 126 of the Sequence Listing) encoding the LEU1 type polypeptide chain (SEQ ID No. 127 of the Sequence Listing); DNA (SEQ ID No. 128 of the Sequence Listing) encoding the LEU2 type polypeptide chain (SEQ ID No. 129 of the Sequence Listing); and

DNA (SEQ ID No. 130 of the Sequence Listing) encoding the LEU3 type polypeptide chain (SEQ Sequence No. 131 of the Sequence Listing).

The following 10 oligonucleotide PCR primers were synthesized in addition to primer 7ALCN (SEQ ID No. 64 of the Sequence Listing) and primer 7AL1P (SEQ ID No. 55 of the Sequence Listing):

5' - AGGGAGGATG GAGATTGGGT GAGCACAAATG TCACCAGTGG A -3'  
(7ALR2; SEQ ID No. 132);  
5' - ATTGTGCTCA CCCAATCTCC ATCCTCCCTG TCTGCATCT -3'  
(7ALF12; SEQ ID No. 133);  
5' - ATCAACACTT TGGCTGGCCT TGCAAGTGAT GGTGACTCTG TC -3'  
(7ALR33; SEQ ID No. 134);  
5' - CCATCACTTG CAAGGCCAGC CAAAGTGGT ATTATGATGG -3'  
(7ALF2; SEQ ID No. 135);  
5' - AGTTTCGAGA TTGGATGCAG CATAGATGAG GAGTTGGGT GCCTTTCC -3'

(7ALR45; SEQ ID No. 136);  
5' - CCCAAGCTCC TCATCTATGC TGCATCCAAT TTGGAAAGTG GGGTC -3'  
(7ALF33; SEQ ID No. 137);  
5' - TTGGCCGAAC GTTCGAGGAT CCTCGTTACT CTGTTGACAG TAGT -3'  
(7ALR53; SEQ ID No. 138);  
5' - ACTACTGTCA ACAGAGTAAC GAGGATCCTC GAACGTTCGG CCAA -3'  
(7ALF53; SEQ ID No. 139);  
5' - CTCATCTATG CTGCATCAA TTTGGAAAGT GGGATCCCAT CAAGG -3'  
(7ALF34; SEQ ID No. 140); and  
5' - ATTGGATGCA GCATAGATGA GGAGCTTGGG TGCCTGTCCT GGTTC -3'  
(7ALR44; SEQ ID No. 141).

(2) Preparation of DNA encoding LEU1 type light chain

1) Preparation of LEU1 DNA fragment

The LEU1-DNA fragment (SEQ ID No. 126 of the Sequence Listing), encoding the amino acid sequence of SEQ ID No. 127 of the Sequence Listing, was prepared by 2-stage PCR, and was then inserted into a plasmid vector and cloned into *E. coli*.

First stage PCR

The outline of the first stage PCR for the preparation of LEU1-DNA is shown in Figure 50.

The LEUA21-DNA fragment, encoding a secretion signal sequence and a portion of the FRL<sub>1</sub> region altered to contain a Hind III restriction enzyme cleavage site at the 5'-end, was prepared as follows. The plasmid pHSGHM17 prepared in Reference Example 14 was used as a template for PCR.

Composition of the PCR reaction solution:

plasmid pHSGHM17 DNA, 25 ng;  
primer 7AL1P, 5 pmol;  
primer 7ALR2, 5 pmol;

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2.5mM dNTP cocktail, 5 µl;  
10 x Pfu buffer, 5 µl;  
Pfu DNA polymerase, 1 unit; and  
redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The LEUB21-DNA fragment, encoding a portion of the FRL<sub>1</sub> region and a portion of the CDRL<sub>1</sub> region, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pKISp35 DNA, 25 ng;  
primer 7ALF12, 5 pmol;  
primer 7ALR33, 5 pmol  
2.5 mM dNTP cocktail, 5 µl;  
10 x Pfu buffer, 5 µl;  
Pfu DNA polymerase, 1 unit; and  
redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The LEUC31 DNA fragment, encoding a portion of the FRL<sub>1</sub> region, the CDRL<sub>1</sub> region, the FRL<sub>2</sub> region and the CDRL<sub>2</sub> region was prepared as follows.

Composition of the PCR reaction solution :

plasmid pHSGHM17 DNA, 25 ng;  
primer 7ALF2, 5 pmol,  
primer 7ALR45 5 pmol,  
2.5mM dNTP cocktail, 5  $\mu$ l;  
10 x Pfu buffer, 5  $\mu$ l;  
Pfu DNA polymerase, 1 unit;  
redistilled water to a final volume of 50  $\mu$ l.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The LEUD21-DNA fragment, encoding a portion of the FRL<sub>2</sub> region, the CDRL<sub>2</sub> region, the FRL<sub>3</sub> region, the CDRL<sub>3</sub> region and a portion of the FRL<sub>4</sub> region was prepared as follows.

Composition of the PCR reaction solution:

plasmid pKISp35 DNA 25 ng;  
primer 7ALF33, 5 pmol;  
primer 7ALR53, 5 pmol;  
2.5 mM dNTP cocktail, 5  $\mu$ l;  
10 x Pfu buffer, 5  $\mu$ l;  
Pfu DNA polymerase 1 unit; and  
redistilled water to a final volume of 50  $\mu$ l.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes was repeated 30 times. After completion of this procedure, the reaction solution

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was heated at 72°C for 10 minutes.

The LEUE21-DNA fragment, encoding a portion of the FRL<sub>3</sub> region, the CDRL<sub>3</sub> region, the FRL<sub>4</sub> region and the constant region, altered to have an EcoRI restriction enzyme cleavage site at the 3'-end, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pKISp35 DNA, 50 ng;  
primer 7ALF53, 5 pmol;  
primer 7ALCN, 5 pmol;  
2.5 mM dNTP cocktail, 5 µl;  
10 x Pfu buffer, 50 µl;  
Pfu DNA polymerase, 1 unit; and  
redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After phenol extraction and ethanol precipitation of each of the PCR-amplified products, each resulting DNA precipitate was separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the acrylamide gel was stained with a 1 µg/ml of ethidium bromide to allow detection of DNA under UV light. The DNA bands corresponding to LEUA21-DNA, LEUB21-DNA, LEUC31-DNA, LEUD21-DNA and LEUE21-DNA were excised with a razor blade.

#### Second stage PCR

The outline of the second stage PCR for the production of LEU1-DNA is shown in Figure 51.

LEU1-DNA, in which the LEUA21-DNA, LEUB21-DNA, LEUC31-DNA, LEUD21-DNA and LEUE21-DNA fragments described above were fused, was prepared as follows.

The electrophoresed gel excised in the first stage PCR was added to the reaction solution without extraction.

Composition of the PCR reaction:

gel piece containing LEUA21-DNA;  
gel piece containing LEUB21-DNA;  
gel piece containing LEUC31-DNA;  
gel piece containing LEUD21-DNA;  
gel piece containing LEUE21-DNA;  
primer 7AL1P, 10 pmol;  
primer 7ALCN, 10 pmol;  
dNTP cocktail, 10 µl;  
10 x Pfu buffer, 10 µl;  
Pfu DNA polymerase, 2 units; and  
redistilled water to a final volume of 100 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

Phenol extraction followed by ethanol precipitation were performed on the amplified PCR fragments, and these fragments were then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide, and viewed under UV light. The band corresponding to LEU1-DNA was excised with a razor blade from the gel, and DNA was eluted from the excised band using Centricon and

a Centrifiruter. The thus eluted DNA was concentrated, first by centrifugation at 7,500 x g and then by ethanol precipitation, after which it was dissolved in 50 µl of distilled water.

## 2) Preparation of plasmid

The LEU1-DNA fragment obtained in 1) above was further purified by phenol extraction followed by ethanol precipitation, and was then digested with the restriction enzymes Hind III and EcoRI. One µg of cloning plasmid pHSG399 DNA (Takara Shuzo Co., Ltd.) was digested with the restriction enzymes Hind III and EcoRI, and then dephosphorylated with CIP. The resulting, dephosphorylated plasmid pHSG399 DNA and the digested LEU1-DNA fragment were ligated using a DNA Ligation Kit Version 2.0 (Takara Shuzo Co., Ltd.). The ligated DNA was then used to transform *E. coli* JM 109 (Takara Shuzo Co., Ltd.). Cells were plated onto LB agar medium containing final concentrations of 0.1 mM IPTG, 0.1% X-Gal and 50 µg/ml chloramphenicol, and the plates were incubated at 37°C overnight to obtain *E. coli* transformants. Any white transformants obtained were cultured in 2 ml of liquid LB medium containing 50 µg/ml chloramphenicol at 37°C overnight, and plasmid DNA was extracted from the resulting cultures by the alkaline-SDS method [c.f. Sambrook et al., supra].

The resulting, extracted plasmid DNA was digested with the restriction enzymes Hind III and EcoRI, and a clone carrying the LEU1-DNA fragment was then identified and selected by 1% w/v agarose gel electrophoresis, stained with ethidium bromide.

Plasmid pHSGLEU15-29-1, carrying DNA encoding a fusion polypeptide of the variable region of the LEU1 type humanized light chain and the constant region of human immunoglobulin κ chain, was obtained accordingly. The transformant *E. coli* pHSGLEU15-29-1 SANK 72598 harboring plasmid pHSGLEU15-29-1 was

deposited with the Kogyo Gijutsuin Seimeい-Kogaku Kogyo Gijutsu Kenkyujo on September 18, 1998, in accordance with the Budapest Treaty, and was accorded the accession number FERM BP-6512.

3) Construction of expression vector

The outline of the method of construction of the expression vector plasmid carrying the DNA encoding the LEU-1 type humanized light polypeptide obtained in the above 2) is shown in Figure 56.

One  $\mu$ g of the plasmid pSRPDHM prepared in Reference Example 23 was digested with the restriction enzymes Hind III and EcoRI, and then dephosphorylated using CIP. The resulting dephosphorylated pSRPDHM DNA (100 ng) was ligated with 10  $\mu$ g of the pHSGLEU15-29-1 DNA fragment which had also been digested with Hind III and EcoRI, using a DNA Ligation Kit, and then used to transform *E. coli* JM109. Cells were then plated on LB agar plates containing 50  $\mu$ g/ml ampicillin and incubated at 37°C.

The transformants obtained by this method were cultured in 2 ml of liquid LB medium containing 50  $\mu$ g/ml ampicillin, and plasmid DNA was subsequently extracted from the resulting culture by the alkaline-SDS method [Sambrook et al., *supra*]. The extracted plasmid DNA was digested with Hind III and EcoRI, and subjected to 1% w/v agarose gel electrophoresis to confirm the presence or absence of the insert of interest, by staining with ethidium bromide. This enabled the identification and isolation of the plasmid pLEUX15-29-5, containing DNA encoding the LEU1 type humanized light chain located downstream of the SR $\alpha$  promoter in the correct orientation.

(3) Preparation of DNA encoding LEU2 type light chain

1) Preparation of LEU2-DNA fragment

The LEU2-DNA fragment (SEQ ID No. 128 of the Sequence Listing), encoding the amino acid sequence of SEQ ID No. 129 of the Sequence Listing, was prepared by two step PCR. The resulting PCR product was then inserted into a plasmid vector and cloned into *E. coli*. The plasmid pHSGHM17, constructed in Reference Example 14, was used as a template for PCR.

First stage PCR

The outline of the first stage PCR for the preparation of LEU2-DNA is shown in Figure 52.

The LEUA21-DNA fragment, LEUB21-DNA fragment, LEUD21-DNA fragment and LEUE21-DNA fragment were each amplified by performing PCR in accordance with the procedure described (1) above, respectively.

The LEUC211-DNA fragment, encoding a portion of the FRL<sub>1</sub> region, the CDRL<sub>1</sub> region, the FRL<sub>2</sub> region and a portion of the CDRL<sub>2</sub> region was prepared as follows.

Composition of the PCR reaction solution:

plasmid pHSGHM17 DNA, 25 ng;  
primer 7ALF2, 5 pmol;  
primer 7ALR44, 5 pmol;  
2.5mM dNTP cocktail, 5 µl;  
10 x Pfu buffer, 5 µl;  
Pfu DNA polymerase, 1 unit; and  
redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for

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1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After phenol extraction and ethanol precipitation of each PCR-amplified product, the resulting DNA precipitate was separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the acrylamide gel was stained with 1 µg/ml of ethidium bromide to allow detection of DNA under UV light. The DNA bands corresponding to LEUA21-DNA, LEUB21-DNA, LEUC211-DNA, LEUD21-DNA and LEUE21-DNA were excised using a razor blade.

#### Second stage PCR

The outline of the second stage PCR for the production of LEU2-DNA fragment is shown in Figure 53.

The LEU2-DNA fragment, in which the LEUA21-DNA, LEUB21-DNA, LEUC211-DNA, LEUD21-DNA and LEUE21-DNA fragment were fused, was prepared as follows.

The electrophoresed gel excised in the first stage PCR was added to the reaction solution without extraction.

The composition of the PCR reaction was as follows:

- gel piece containing LEUA21-DNA;
- gel piece containing LEUB21-DNA;
- gel piece containing LEUC211-DNA;
- gel piece containing LEUD21-DNA;
- gel piece containing LEUE21-DNA;
- primer 7AL1P, 10 pmol;
- primer 7ALCN, 10 pmol;
- dNTP cocktail, 10 µl;
- 10 x Pfu buffer, 10 µl;
- Pfu DNA polymerase, 2 units; and

redistilled water to a final volume of 100  $\mu$ l.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

Phenol extraction and then ethanol precipitation were performed on the amplified PCR fragments, and these fragments were then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the acrylamide gel was stained with 1  $\mu$ g/ml of ethidium bromide, and analyzed under UV light. The gel at the band corresponding to LEU2-DNA was excised using a razor blade, and DNA was eluted from the gel using Centricon and a Centrifiruter. The eluted DNA was concentrated first by centrifugation at 7,500  $\times g$ , precipitated with ethanol, then dissolved in 50  $\mu$ l of distilled water.

## 2) Preparation of plasmid

The LEU2-DNA fragment obtained in 1) above was further purified by phenol extraction followed by ethanol precipitation, and it was then digested with the restriction enzymes Hind III and EcoRI. One  $\mu$ g of cloning plasmid pHSG399 DNA was digested with the restriction enzymes Hind III and EcoRI, before being dephosphorylated with CIP. The resulting, dephosphorylated plasmid pHSG399 DNA and the digested LEU2-DNA fragment were ligated using a DNA Ligation Kit. The ligated DNA was then used to transform *E. coli* JM 109. The cells were plated onto LB agar medium containing final concentrations of 0.1 mM IPTG, 0.1% X-Gal and 50  $\mu$ g/ml chloramphenicol. Any white transformants obtained were cultured in 2 ml of liquid LB medium containing 50  $\mu$ g/ml

chloramphenicol, and plasmid DNA was extracted from the resulting culture by the alkaline-SDS method. The resulting plasmid DNA was digested with the restriction enzymes Hind III and EcoRI, and a clone carrying the LEU2-DNA fragment was then selected after analysis by 1% w/v agarose gel electrophoresis, stained with ethidium bromide.

Plasmid pHSGLEU21-28-8 carrying DNA, encoding a fusion polypeptide of the variable region of the LEU2 type humanized light chain and the constant region of human immunoglobulin κ chain, was obtained accordingly. The transformant *E. coli* pHSGLEU21-28-8 SANK 72698, harboring plasmid pHSGLEU21-28-8, was deposited with the Kogyo Gijutsuin Seimeい-Kogaku Kogyo Gijutsu Kenkyujo on September 18, 1998, in accordance with the Budapest Treaty, and was accorded the accession number FERM BP-6511.

### 3) Construction of expression vector

The outline of the method of construction of the expression vector plasmid carrying the DNA encoding the LEU-2 type humanized light polypeptide obtained in the above (2) is shown in Figure 56.

One μg of the plasmid pSRPDHM prepared in Reference Example 23 was digested with the restriction enzymes Hind III and EcoRI, and then dephosphorylated using CIP. The resulting dephosphorylated plasmid DNA (100 ng) was ligated with 10 μg of the pHSGLEU21-28-8 DNA fragment which had also been digested with Hind III and EcoRI, using a DNA Ligation Kit. The resulting ligated DNA was then used to transform *E. coli* JM109. Cells were then plated on LB agar plates containing 50 μg/ml ampicillin, followed by incubation at 37°C.

The transformants obtained by this method were cultured in

liquid LB medium containing 50 µg/ml ampicillin, and plasmid DNA was subsequently extracted from the resulting culture by the alkaline-SDS method. The extracted plasmid DNA was digested with Hind III and EcoRI, and subjected to analysis on a 1% w/v agarose gel and, after electrophoresis, to confirm the presence or absence of the insert of interest by staining with ethidium bromide and observation under UV light. This enabled the isolation of the plasmid pLEUX22-7-1, which contains DNA encoding the LEU2 type humanized light chain located downstream of the SRα promoter in the correct orientation.

(4) Preparation of DNA encoding LEU3 type light chain

1) Preparation of LEU3-DNA fragment

The LEU3-DNA fragment (SEQ ID No. 130 of the Sequence Listing), encoding the amino acid sequence of SEQ ID No. 131 of the Sequence Listing, was prepared by performing a two step PCR. The product thus obtained was then inserted into a plasmid vector and cloned into *E. coli*. The plasmid pHSGHM17 constructed in Reference Example 14 was used as a template for PCR.

First stage PCR

The outline of the first stage PCR for the preparation of LEU3-DNA fragment is shown in Figure 54.

The LEUA21-DNA fragment, the LEUB21-DNA fragment, the LEUC31-DNA fragment and the LEUE21-DNA fragment were amplified by performing PCR in accordance with the procedure as described (1), respectively.

The LEUD31-DNA fragment, encoding a portion of the FRL<sub>2</sub> region, the CDRL<sub>2</sub> region, the FRL<sub>3</sub> region, the CDRL<sub>3</sub> region and a portion of the FRL<sub>4</sub> region was prepared as follows:

Composition of the PCR reaction solution:

plasmid pKISp35 DNA, 25 ng;  
primer 7ALF34, 5 pmol;  
primer 7ALR53, 5 pmol;  
2.5 mM dNTP cocktail, 5 µl;  
10 x Pfu buffer, 5 µl;  
Pfu DNA polymerase, 1 unit; and  
redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After phenol extraction and ethanol precipitation of each PCR-amplified product, the resulting DNA precipitate was separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the acrylamide gel was stained with 1 µg/ml of ethidium bromide to allow detection of DNA under UV light. The gel at the bands corresponding to LEUA21-DNA, LEUB21-DNA, LEUC31-DNA, LEUD31-DNA and LEUE21-DNA was excised, using a razor blade.

#### Second stage PCR

The outline of the second stage PCR for the production of LEU3-DNA fragment is shown in Figure 55.

The LEU3-DNA fragment, in which the LEUA21-DNA, LEUB21-DNA, LEUC31-DNA, LEUD31-DNA and LEUE21-DNA fragments, were fused, was prepared as follows.

The electrophoresed gel excised in the first stage PCR was added to the reaction solution without extraction.

Composition of the PCR reaction:

gel piece containing LEUA21-DNA;  
gel piece containing LEUB21-DNA;  
gel piece containing LEUC31-DNA;  
gel piece containing LEUD31-DNA;  
gel piece containing LEUE21-DNA;  
primer 7AL1P, 10 pmol;  
primer 7ALCN, 10 pmol;  
dNTP cocktail, 10 µl;  
10 x Pfu buffer, 10 µl;  
Pfu DNA polymerase, 2 units; and  
redistilled water to a final volume of 100 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

Phenol extraction and then ethanol precipitation were performed on the amplified PCR fragments, and these fragments were then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide, and analyzed under UV light. The gel at the band corresponding to LEU3-DNA was excised, using a razor blade, and DNA was eluted from the gel using Centricon and a Centrifiruter. The eluted DNA was concentrated first by centrifugation at 7,500 x g, then precipitated with ethanol, after which it was dissolved in 50 µl of distilled water.

## 2) Preparation of plasmid

The LEU3-DNA fragment obtained in 1) above was further purified by phenol extraction followed by ethanol precipitation, and it was then digested with the restriction enzymes Hind III and EcoRI. One µg of cloning plasmid pHSG399 DNA was digested with the restriction enzymes Hind III and EcoRI, and then dephosphorylated with CIP. The resulting, dephosphorylated plasmid pHSG399 DNA and the digested LEU3-DNA fragment were ligated using a DNA Ligation Kit (Version 2.0, Takara Shuzo Co., Ltd.).

The ligated DNA was then used to transform *E. coli* JM 109. The cells were plated onto LB agar medium containing final concentrations of 0.1 mM IPTG, 0.1% X-Gal and 50 µg/ml chloramphenicol, and the plates were incubated at 37°C to obtain *E. coli* transformants. Any white transformants obtained were cultured in a liquid LB medium containing 50 µg/ml chloramphenicol, and plasmid DNA was extracted from the resulting culture by the alkaline-SDS method [Sambrook et al., *supra*]. The resulting, extracted plasmid DNA was digested with the restriction enzymes Hind III and EcoRI, and a clone carrying the LEU3-DNA fragment was then identified and selected by 1% w/v agarose gel electrophoresis, stained with ethidium bromide.

Plasmid pHSGLEU31-6-2 carrying DNA, encoding a fusion polypeptide of the variable region of the LEU3 type humanized light chain and the constant region of human immunoglobulin κ chain, was obtained accordingly. The transformant *E. coli* pHSGLEU31-6-2 SANK 72798, harboring plasmid pHSGLEU31-6-2, was deposited with the Kogyo Gijutsuin Seimeい-Kogaku Kogyo Gijutsu Kenkyujo on September 18, 1998, in accordance with the Budapest Treaty, and was accorded the accession number FERM BP-6513.

### 3) Construction of expression vector

The outline of the method of construction of the expression vector plasmid, carrying the DNA encoding the LEU-3 type humanized light polypeptide, obtained in 2) above, is shown in Figure 56.

One µg of the plasmid pSRPDHM, prepared in Reference Example 23, was digested with the restriction enzymes Hind III and EcoRI, and then dephosphorylated using CIP. 100 ng of the dephosphorylated pSRPDHM DNA was ligated with LEU3-DNA which had also been digested with Hind III and EcoRI, using a DNA Ligation Kit, and the ligation product was then used to transform *E. coli* JM109. Cells were then plated on LB agar plates containing 50 µg/ml ampicillin and cultured at 37 °C.

The transformants obtained by this method were cultured in liquid LB medium containing 50 µg/ml ampicillin, and plasmid DNA was subsequently extracted from the resulting culture by the alkaline-SDS method. The extracted plasmid DNA was digested with Hind III and EcoRI, and subjected to 1% w/v agarose gel electrophoresis, stained with ethidium bromide, to confirm the presence or absence of the insert of interest. This enabled the isolation of the plasmid pLEUX31-6-2, which contains DNA encoding the LEU3 type humanized light chain, located downstream of the SRα promoter in the correct orientation.

#### (5) Verification of the nucleotide sequences

To verify that the DNA inserts of plasmids obtained in the above (2) to (4) have the desired nucleotide sequences, their DNA inserts were analyzed to determine the nucleotide sequences, using primers 7ALLP and 7ALCN as sequencing primers.

As a result, it was established that the plasmids pLEUX15-29-5, pLEUX22-7-1 and pLEUX31-6-2 had the nucleotide sequences SEQ ID No. 126, SEQ ID No. 128; and SEQ ID No. 130, respectively, of the Sequence Listing.

### EXAMPLE 3

#### Construction of Expression Vector of Eu type Humanized Heavy Chain

The heavy chain of the HFE7A antibody, humanized using 8E10 prepared in Reference Example 15 as an acceptor, was modified by using PCR primers so that the 8E10 FR region was substituted by the Eu type, in order to prepare an Eu type humanized heavy chain as described below (Figure 49).

The 44th amino acid (Arg) and the 76th amino acid (Ala) from the N-terminus of the amino acid sequence of the heavy chain of the humanized HFE7A were replaced with Gly and Thr respectively, which are present in Eu. The 113th amino acid (Glu) was replaced with Gln which is conserved in the human Ig heavy chain subgroup I. The resulting amino acid sequence was designated as "HEU1 type".

The 44th amino acid (Arg) and the 76th amino acid (Ala) from the N-terminus were replaced with Gly and Thr, respectively, and the 113th amino acid (Glu) was replaced with Gln, which is conserved in the human H chain subgroup I. The 70th amino acid (Leu) was replaced with Ile which is present in Eu. The resulting amino acid sequence is referred to as "HEU2 type".

The 44th amino acid (Arg) and the 76th amino acid (Ala) from the N-terminus of the amino acid sequence of the heavy chain of the humanized HFE7A prepared in Reference Example 15 (SEQ ID No.

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89 of the Sequence Listing) were replaced with Gly and Thr, respectively, the 113th amino acid (Glu) was replaced with Gln and the 38th amino acid (Lys) was replaced with Arg, which is present in Eu. The resulting amino acid sequence is referred to as "HEU3 type".

Expression plasmids, respectively carrying these 3 types of HEU type humanized heavy chain amino acid sequences from the anti-Fas antibody, were constructed as follows.

#### (1) Synthesis of primers

PCR was used to construct the following DNA sequences encoding each type of HEU type humanized heavy chains designed above:

DNA (SEQ ID No. 142 of the Sequence Listing) encoding the HEU1 type heavy chain (SEQ ID No. 143 of the Sequence Listing); DNA (SEQ ID No. 144 of the Sequence Listing) encoding the HEU2 type heavy chain (SEQ ID No. 145 of the Sequence Listing); and DNA (SEQ ID No. 146 of the Sequence Listing) encoding the HEU3 type heavy chain (SEQ Sequence No. 147 of the Sequence Listing).

The following oligonucleotide PCR primers were synthesized:

5' - CCAAGCTTGG CTTGACCTCA CCATGGGATG GAGCTGTA -3'

(7AH1P; SEQ ID No. 148);

5' - AGTGGGTAAA ACAGGCCCT GGACAGGGAC TTGAGTGGAT -3'

(HEU16F; SEQ ID No. 149);

5' - ATCCACTCAA GTCCCTGTCC AGGGGCCTGT TTTACCCACT -3'

(HEU16R; SEQ ID No. 150);

5' - AAGACCGATG GGCCCTTGGT GGAGGCTGAG GAGACGGTGA CCAGTGTACC

TTGGCCCCAG ACAT -3'

(HEU28R; SEQ ID No. 151);

5' - GTTCAAGGGC AAGGCCACAA TAACTGTAGA CACATCCGC -3'

(HEU25F; SEQ ID No. 152);

5' - GCGGATGTGT CTACAGTTAT TGTGGCCTTG CCCTTGAAC -3'

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(HEU25R; SEQ ID No. 153) ;  
5' - AGTGGGTACG ACAGGCCCT GGACAAGGAC TTGAGTGGAT -3'  
(HEU36F; SEQ ID No. 154); and  
5' - ATCCACTCAA GTCCTTGTCC AGGGGCCTGT CGTACCCACT-3'  
(HEU36R; SEQ ID No. 155).

(2) Preparation of DNA encoding HEU1 type heavy chain

1) Preparation of HEU1-DNA fragment

The HEU1HA-DNA fragment, containing the nucleotide sequence encoding the amino acids 1 to 125 of SEQ ID No. 143 of the Sequence Listing, was prepared by two step PCR. The product was then inserted into a plasmid vector and cloned into *E. coli*. The plasmid pgHSL7A62, prepared in Reference Example 15, was used as the template for PCR.

First stage PCR

The outline of the first stage PCR for the preparation of HEU1HA-DNA is shown in Figure 57.

The HEU161A-DNA fragment, encoding a secretion signal sequence, the FRH<sub>1</sub> region, the CDRH<sub>1</sub> region and a portion of the FRH<sub>2</sub> region altered to contain a Hind III restriction enzyme cleavage site at the 5'-end, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pgHSL7A62 DNA, 50 ng;  
primer 7AH1P, 5 pmol;  
primer HEU16R, 5 pmol;  
2.5mM dNTP cocktail, 5 µl;  
10 x Pfu buffer, 5 µl;  
Pfu DNA polymerase, 1 unit; and  
redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The HEU161B2-DNA fragment, encoding the FRH<sub>2</sub> region, the CDRH<sub>2</sub> region, the FRH<sub>3</sub> region, the CDRH<sub>3</sub> region, the FRH<sub>4</sub> region and a portion of the constant region of human Ig heavy chain was prepared as follows.

Composition of the PCR reaction solution:

plasmid pgHSL7A62 DNA, 50 ng;  
primer HEU16F, 5 pmol;  
primer HEU28R, 5 pmol;  
2.5 mM dNTP cocktail, 5 µl;  
10 x Pfu buffer, 5 µl;  
Pfu DNA polymerase, 1 unit; and  
redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After phenol extraction and ethanol precipitation of each PCR-amplified product, the resulting DNA precipitate was separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the acrylamide gel was stained with a 1 µg/ml solution of ethidium bromide to allow detection of DNA under UV light. The DNA bands corresponding to HEU161A-DNA and HEU161B2-DNA were excised using a razor blade.

Second stage PCR

The outline of the second stage PCR for the production of HEU1HA-DNA is shown in Figure 58.

HEU1HA-DNA fragment, in which the HEU161A-DNA and HEU161B2-DNA fragments described above were fused, was prepared as follows. The electrophoresed gel excised in the first stage PCR was added to the reaction solution without extraction.

## Composition of the PCR reaction:

gel piece containing HEU161A-DNA  
gel piece containing HEU161B2-DNA  
primer 7AH1P, 5 pmol;  
primer HEU28R, 5 pmol;  
2.5mM dNTP cocktail, 5 µl;  
10 x Pfu buffer, 5 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

Phenol extraction and then ethanol precipitation were performed on the amplified PCR fragments, and these fragments were then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide, and analyzed under UV light. The gel at the position of the band corresponding to HEU1HA-DNA was excised, using a razor blade, and DNA was eluted from the gel using Centricon and a Centrifiruter. The eluted DNA was concentrated

first by centrifugation at 7,500 × g, precipitated with ethanol, then dissolved in 50 µl of distilled water.

## 2) Preparation of plasmid

The HEU1HA-DNA fragment, obtained in 1) above, was further purified by phenol extraction followed by ethanol precipitation, and it was then digested with the restriction enzymes Hind III and ApaI. One µg of plasmid pgHSL7A62 DNA was digested with the restriction enzymes Hind III and ApaI, and then dephosphorylated with CIP. The resulting, dephosphorylated plasmid pgHSL7A62 DNA and the digested HEU1HA-DNA fragment were ligated using a DNA Ligation Kit (Version 2.0, Takara Shuzo Co., Ltd.). The ligated DNA was then used to transform *E. coli* JM 109.

The cells were plated onto LB agar medium containing final concentrations of 50 µg/ml chloramphenicol, and the plates were incubated at 37°C to obtain *E. coli* transformants. The transformants obtained were cultured in liquid LB medium containing 50 µg/ml chloramphenicol, and plasmid DNA was extracted from the resulting culture by the alkaline-SDS method [Sambrook et al., *supra*]. The resulting, extracted plasmid DNA was digested with the restriction enzymes Hind III and ApaI, and a clone carrying the HEU1HA-DNA fragment was then selected by agarose gel electrophoresis, stained with ethidium bromide.

Plasmid pHSGAB580-3-21, carrying DNA encoding a fusion polypeptide of the variable region of the HEU1 type humanized heavy chain and the constant region of human IgG1 was obtained accordingly. The transformant *E. coli* pHSGAB580-3-21 SANK 72898, harboring plasmid pHSGAB580-3-21, was deposited with the Kogyo Gijutsuin Seimei-Kogaku Kogyo Gijutsu Kenkyujo on September 18, 1998, in accordance with the Budapest Treaty, and was accorded the accession number FERM BP-6515.

### 3) Construction of expression vector

The outline of the method of construction of the expression vector plasmid carrying the DNA encoding the HEU1 type humanized heavy polypeptide obtained in 2) above was shown in Figure 63.

Ten µg of pHSGAB580-3-21 DNA was digested with the restriction enzymes Hind III and ApaI. One µg of the plasmid pSRgPDH prepared in Reference Example 23 was digested with the restriction enzymes Hind III and ApaI, and then dephosphorylated using CIP. 100 ng of the resulting dephosphorylated pSRgPDH was ligated with 10 µg of the HEU1-DNA fragment digested with the restriction enzymes Hind III and ApaI, using a DNA Ligation Kit (Version 2.0, Takara Shuzo Co., Ltd.), and then used to transform *E. coli* JM109. Cells were then plated on LB agar plates containing 50 µg/ml ampicillin, and cultured at 37°C.

The transformants obtained by this method were cultured in liquid LB medium containing 50 µg/ml ampicillin, and plasmid DNA was subsequently extracted from the resulting culture by the alkaline-SDS method [Sambrook et al., *supra*]. The extracted plasmid DNA was digested with Hind III and ApaI, and subjected to 1% w/v agarose gel electrophoresis, stained with ethidium bromide, to confirm the presence or absence of the insert of interest. This enabled the isolation of the plasmid pHEUX 580-3-23, which contains DNA encoding the HEU1 type humanized heavy chain located downstream of the SRα promoter, and in the correct orientation.

### (3) Preparation of DNA encoding HEU2 type heavy chain

#### 1) Preparation of HEU2-DNA fragment

The HEU2HA-DNA fragment containing the nucleotide sequence encoding amino acids 1-125 of SEQ ID No. 145 of the Sequence Listing was prepared by performing a two step PCR. The product

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was then inserted into a plasmid vector and cloned into *E. coli*. The plasmid pgHSL7A62 constructed in Reference Example 15 was used as a template for PCR.

#### First stage PCR

The outline of the first stage PCR for the preparation of HEU2HA-DNA fragment is shown in Figure 59.

The HEU261A-DNA fragment, encoding a secretion signal sequence, the FRH<sub>1</sub> region, the CDRH<sub>1</sub> region and a portion of the FRH<sub>2</sub> region altered to contain a Hind III restriction enzyme cleavage site at the 5'-end, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pgHSL7A62 DNA, 50 ng;  
primer 7AH1P, 5 pmol;  
primer HEU16R, 5 pmol;  
2.5 mM dNTP cocktail, 5 µl;  
10 x Pfu buffer, 5 µl;  
Pfu DNA polymerase, 1 unit; and  
redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The HEU261B-DNA fragment, encoding the FRH<sub>2</sub> region, the CDRH<sub>2</sub> region and a portion of the FRH<sub>3</sub> region was prepared as follows.

Composition of the PCR reaction solution:

plasmid pgHSL7A62 DNA, 50 ng;

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primer HEU16F, 5 pmol;  
primer HEU25R, 5 pmol;  
2.5 mM dNTP cocktail, 5 µl;  
10 x Pfu buffer, 5 µl;  
Pfu DNA polymerase, 1 unit; and  
redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

The HEU261C2-DNA fragment, encoding a portion of the CDRH<sub>2</sub> region, the FRH<sub>3</sub> region, the CDRH<sub>3</sub> region, the FRH<sub>4</sub> region and a portion of constant region of human Ig heavy chain was prepared as follows.

Composition of the PCR reaction solution:

plasmid pgHSL7A62 DNA, 50 ng;  
primer HEU25F, 5 pmol;  
primer HEU28R, 5 pmol;  
2.5 mM dNTP cocktail, 5 µl;  
10 x Pfu buffer, 5 µl;  
Pfu DNA polymerase (Stratagene), 1 unit; and  
redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

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After phenol extraction and ethanol precipitation of each PCR-amplified product, the resulting DNA precipitate was separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the acrylamide gel was stained with 1 µg/ml of ethidium bromide to allow detection of DNA under UV light. The DNA bands corresponding to HEU261A-DNA, HEU261B-DNA and HEU261C2-DNA were excised, using a razor blade.

#### Second stage PCR

The outline of the second stage PCR for the production of HEU2HA-DNA fragment is shown in Figure 60.

HEU2HA-DNA fragment, in which the HEU261A-DNA, HEU261B-DNA and HEU261C2-DNA fragments were fused, was prepared as follows. The electrophoresed gel excised in the first stage PCR was added to the reaction solution without extraction.

#### Composition of the PCR reaction:

gel piece containing HEU261A-DNA;  
gel piece containing HEU261B-DNA;  
gel piece containing HEU261C2-DNA;  
primer 7AH1P, 5 pmol;  
primer HEU28R, 5 pmol;  
2.5 mM dNTP cocktail, 5 µl;  
10 x Pfu buffer, 5 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

Phenol extraction and then ethanol precipitation were performed on the amplified PCR fragments, and these fragments were then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1 µg/ml of ethidium bromide, and observed under UV light. The gel at the position of the band corresponding to HEU2HA-DNA was excised, using a razor blade, and DNA was eluted from the gel using Centricon and a Centrifiruter. The eluted DNA was concentrated first by centrifugation at 7,500 × g, then precipitated with ethanol, and finally dissolved in 50 µl of distilled water.

## 2) Preparation of plasmid

The HEU2HA-DNA fragment obtained above 1) was further purified by phenol extraction followed by ethanol precipitation, and it was then digested with the restriction enzymes Hind III and ApaI. One µg of plasmid pgHSL7A62 DNA was digested with the restriction enzymes Hind III and ApaI, and then dephosphorylated with CIP. The resulting, dephosphorylated plasmid pgHSL7A62 DNA and the HEU2HA-DNA fragment, digested with the restriction enzymes, were ligated using a DNA Ligation Kit (Version 2.0, Takara Shuzo Co., Ltd.). The ligated DNA then used to transform *E. coli* JM 109. The cells were plated onto LB agar medium containing final concentrations of 50 µg/ml chloramphenicol, and were cultured at 37°C. The resulting transformants were cultured in liquid LB medium containing 50 µg/ml chloramphenicol, and plasmid DNA was extracted from the resulting culture by the alkaline-SDS method [Sambrook et al., *supra*]. The resulting, extracted plasmid DNA was digested with the restriction enzymes Hind III and ApaI, and a clone carrying the HEU2HA-DNA fragment was then selected by agarose gel electrophoresis with ethidium bromide staining.

There was thus obtained plasmid pHSGHEU223-30-1, carrying the fragment of DNA encoding the polypeptide in which the

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variable region of the HEU2 type humanized heavy chain and DNA encoding the constant region of human IgG1, are ligated. The transformant *E. coli* pHSGHEU223-30-1 SANK 72998, harboring plasmid pHSGHEU223-30-1, was deposited with the Kogyo Gijutsuin Seimei-Kogaku Kogyo Gijutsu Kenkyujo on September 18, 1998, in accordance with the Budapest Treaty, and was accorded the accession number FERM BP-6516.

### 3) Construction of expression vector

The outline of the method of construction of the expression vector plasmid carrying the DNA encoding the HEU-2 type humanized heavy polypeptide obtained in 2) above is shown in Figure 63.

Ten µg of the pHSGHEU223-30-1 was digested with the restriction enzymes Hind III and ApaI. One µg of the plasmid pSRgPDH, prepared in Reference Example 23, was digested with the restriction enzymes Hind III and ApaI, and then dephosphorylated using CIP. 100 ng of the resulting dephosphorylated plasmid DNA was ligated with 10 µg of the HEU2-DNA fragment digested with Hind III and ApaI, using a DNA Ligation Kit, and then used to transform *E. coli* JM109. Cells were then plated on LB agar plates containing 50 µg/ml ampicillin, and incubated at 37°C.

The transformants obtained by this method were cultured in liquid LB medium containing 50 µg/ml ampicillin, and plasmid DNA was subsequently extracted from the resulting culture by the alkaline-SDS method [Sambrook et al., *supra*]. The extracted plasmid DNA was digested with Hind III and ApaI, and subjected to 1% w/v agarose gel electrophoresis, with ethidium bromide staining, to confirm the presence or absence of the insert of interest. This enabled the isolation of the plasmid pHEUX222-1-4, which contains DNA encoding the HEU2 type humanized heavy chain, located downstream of the SRα promoter, in the correct orientation.

(4) Preparation of DNA encoding HEU3 type heavy chain

1) Preparation of HEU3-DNA fragment

The HEU3HA-DNA fragment, containing the nucleotide sequence encoding amino acids 1 to 125 sequence of SEQ ID No. 147 of the Sequence Listing, was prepared by performing two stage PCR. The product was then inserted into a plasmid vector and cloned into *E. coli*. The plasmid pgHSL7A62, constructed in Reference Example 15, was used as a template for PCR.

First stage PCR

The outline of the first stage PCR for the preparation of HEU3HA-DNA is shown in Figure 61.

The HEU361A-DNA fragment, encoding a secretion signal sequence, the FRH<sub>1</sub> region, the CDRH<sub>1</sub> region and a portion of the FRH<sub>2</sub> region altered to contain a Hind III restriction enzyme cleavage site at the 5'-end, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pgHSL7A62 DNA, 50 ng;  
primer 7AH1P, 5 pmol;  
primer HEU36R, 5 pmol;  
2.5 mM dNTP cocktail, 5 µl;  
10 x Pfu buffer, 5 µl;  
Pfu DNA polymerase, 1 unit; and  
redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction

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solution was heated at 72°C for 10 minutes.

The HEU361B2-DNA fragment, encoding the FRH<sub>2</sub> region, the CDRH<sub>2</sub> region, the FRH<sub>3</sub> region, the CDRH<sub>3</sub> region, the FRH<sub>4</sub> region and a portion of the constant region of the human Ig heavy chain was prepared as follows.

Composition of the PCR reaction solution:

- plasmid pgHSL7A62 DNA, 50 ng;
- primer HEU36F, 5 pmol;
- primer HEU28R, 5 pmol;
- 2.5 mM dNTP cocktail, 5 µl;
- 10 × Pfu buffer, 5 µl;
- Pfu DNA polymerase, 1 unit; and
- redistilled water to a final volume of 50 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After phenol extraction and ethanol precipitation of each PCR-amplified product, the resulting DNA precipitate was separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the acrylamide gel was stained with 1 µg/ml of ethidium bromide to allow detection of DNA under UV light. The gel at the bands corresponding to HEU361A-DNA and HEU361B2-DNA was excised, using a razor blade.

#### Second stage PCR

The outline of the second stage PCR for the production of HEU3HA-DNA fragment is shown in Figure 62.

The HEU3HA-DNA fragment, in which the HEU361A-DNA and HEU361B2-DNA were fused, was prepared as follows. The electrophoresed gel excised in the first stage PCR was added to the reaction solution without extraction.

Composition of the PCR reaction:

gel piece containing HEU361A-DNA;  
gel piece containing HEU361B2-DNA;  
primer 7AH1P, 5 pmol;  
primer HEU28R, 5 pmol;  
2.5mM dNTP cocktail, 5  $\mu$ l;  
10 x Pfu buffer, 5  $\mu$ l;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 50  $\mu$ l.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, after which a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

Phenol extraction and then ethanol precipitation were performed on the amplified PCR fragments, and these fragments were then separated by 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the gel was stained with 1  $\mu$ g/ml of ethidium bromide, and analyzed under UV light. The gel at the position of the band corresponding to HEU3HA-DNA was excised, using a razor blade, and DNA was eluted from the gel using Centricon and a Centrifiruter. The eluted DNA was concentrated first by centrifugation at 7,500  $\times g$ , then precipitated with ethanol, and finally dissolved in 50  $\mu$ l of distilled water.

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## 2) Preparation of plasmid

The HEU3HA-DNA fragment obtained in 1) above was further purified by phenol extraction, followed by ethanol precipitation. The purified fragment was then digested with the restriction enzymes Hind III and ApaI. One µg of plasmid pgHSL7A62 DNA was digested with the restriction enzymes Hind III and ApaI, and then dephosphorylated with CIP. The resulting, dephosphorylated plasmid pgHSL7A62 DNA and the digested HEU3HA-DNA fragment were then ligated using a DNA Ligation Kit (Version 2.0, Takara Shuzo Co., Ltd.). The ligated DNA was then used to transform *E. coli* JM 109. The cells were plated onto LB agar medium containing final concentrations of 50 µg/ml chloramphenicol, and were incubated at 37°C. Resulting transformants were cultured in liquid LB medium containing 50 µg/ml chloramphenicol, and plasmid DNA was extracted from the resulting culture by the alkaline-SDS method [Sambrook et al., *supra*]. The resulting, extracted plasmid DNA was digested with the restriction enzymes Hind III and ApaI, and a clone carrying the HEU3HA-DNA fragment was then selected by agarose gel electrophoresis.

Plasmid pHSGHEU222-1-2 was thus obtained, carrying DNA encoding a polypeptide wherein the variable region of the HEU3 type humanized heavy chain and the constant region of human IgG1 are ligated. The transformant *E. coli* pHSGHEU222-1-2 SANK 73098, harboring plasmid pHSGHEU222-1-2, was deposited with the Kogyo Gijutsuin Seimei-Kogaku Kogyo Gijutsu Kenkyujo on September 18, 1998, in accordance with the Budapest Treaty, and was accorded the accession number FERM BP-6514.

## 3) Construction of expression vector

The outline of the method of construction of the expression vector plasmid carrying the DNA encoding the HEU3 type humanized heavy polypeptide obtained in 2) above is shown in Figure 63.

Ten µg of the plasmid pHSGHEU222-1-2 was digested with the restriction enzymes Hind III and ApaI. One µg of the plasmid pSRgPDH, prepared in Reference Example 23, was digested with the restriction enzymes Hind III and ApaI, and then dephosphorylated using CIP. 100 ng of the dephosphorylated pSRgPDH was ligated with HEU3-DNA which had also been digested with Hind III and ApaI, using a DNA Ligation Kit, and then used to transform *E. coli* JM109. Cells were then plated on LB agar plates containing 50 µg/ml ampicillin and cultured at 37 °C.

Any transformants were cultured in liquid LB medium containing 50 µg/ml ampicillin, and plasmid DNA was subsequently extracted from the resulting culture by the alkaline-SDS method. The extracted plasmid DNA was digested with Hind III and ApaI, and subjected to 1% w/v agarose gel electrophoresis, with ethidium bromide staining, to confirm the presence or absence of the insert of interest. This enabled the isolation of the plasmid pHEUX 322-22-5, which contains DNA, encoding the HEU3 type humanized heavy chain located downstream of the SRα promoter, in the correct orientation.

#### (5) Verification of the nucleotide sequences

To verify that the DNA inserts of plasmids obtained in (2) to (4) above had the desired nucleotide sequences, their DNA inserts were analyzed, in order to determine the nucleotide sequences, using primers 7AH1P and HEU28R as sequencing primers.

As a result, it was established that the DNA inserts of plasmids pHEUX580-3-23, pHEUX222-1-4 and pHEUX322-22-5 had the nucleotide sequences: SEQ ID No.142, SEQ ID No. 144; and SEQ ID No. 146, respectively, of the Sequence Listing.

**EXAMPLE 4****Expression of Eu type Humanized Antibody in COS-7 Cells**

Transfection of COS-7 cells with each of the plasmids prepared above, for expression of Eu type humanized heavy chain and Eu type humanized light chains, constructed in Examples 2 and 3, was effected by a transfection method using the transfection reagent FuGENE6 (Transfection reagent, Boehringer Mannheim).

**2 x 10<sup>5</sup>** COS-7 cells were grown to semi-confluence in a petri dish for cell culture (diameter: 90 mm; culture area: 57 cm<sup>2</sup>; Sumitomo Bakelite, K. K.) containing DMEM (Nissui pharmaceuticals K. K.) supplemented with 10% v/v fetal calf serum (FCS) (Moregate).

Six  $\mu$ l of the transfection reagent was added into 200  $\mu$ l of DMEM which did not contain FCS, and allowed to stand at room temperature for 5 minutes (hereinafter referred to as "transfection reagent/DMEM").

2  $\mu$ g of HEU type humanized heavy chain expression plasmid (pHEUX580-3-23, pHEUX222-1-4 or pHEUX322-22-5) DNA and 2  $\mu$ g of LEU type humanized light chain expression plasmid (pLEUX15-29-5, pLEUX22-7-1 or pLEUX31-6-2) prepared with a large-scale plasmid preparation kit (Withered MaxiPrep DNA Purification System; Promega) were mixed, subjected to ethanol precipitation in the same tube, and then suspended in 5  $\mu$ l of distilled water. 206  $\mu$ l of the transfection reagent/DMEM was then added to the suspension. After 15 minutes, the transfection reagent/DMEM, containing the DNA, was layered over the COS-7 cells in the dish. The cells were then cultured at 37°C for 72 hours under an atmosphere of 5% v/v gaseous CO<sub>2</sub>, after which time the

supernatant was recovered.

Using the above method, COS-7 cells were variously transfected and cultured with each of the following plasmid or plasmid combinations, after which the supernatant was recovered:

- (A) : no plasmid DNA
- (B) : cotransfection with pHEUX580-3-23 and pLEUX15-29-5
- (C) : cotransfection with pHEUX222-1-4 and pLEUX15-29-5
- (D) : cotransfection with pHEUX322-22-5 and pLEUX15-29-5
- (E) : cotransfection with pHEUX580-3-23 and pLEUX22-7-1
- (F) : cotransfection with pHEUX222-1-4 and pLEUX22-7-1
- (G) : cotransfection with pHEUX322-22-5 and pLEUX22-7-1
- (H) : cotransfection with pHEUX580-3-23 and pLEUX31-6-2
- (I) : cotransfection with pHEUX222-1-4 and pLEUX31-6-2
- (J) : cotransfection with pHEUX322-22-5 and pLEUX31-6-2

#### EXAMPLE 5

##### Quantification of Expressed Products by ELISA

Expression of humanized antibody in the culture supernatant prepared in Example 4 was verified, and quantification of the expressed products was performed in accordance with a method similar to that described in Reference Example 17, but wherein DMEM was used as a diluent. Accordingly, it was verified that each of the expression products of culture supernatants [B], [C], [D], [E], [F], [G], [H], [I] and [J], prepared in Example 4 above, was specifically detected by the anti-human IgG antibody.

## EXAMPLE 6

### **Assay for Human Fas-Binding Activity**

The assay for Fas-binding activity in the cell culture supernatant fluids prepared in Example 4 was performed using a similar method to that described in Reference Example 18, but wherein DMEM was used as a diluent. Binding activity for the human Fas fusion protein was demonstrated for the expressed products of culture supernatants of categories (B), (C), (D), (E), (F), (G), (H), (I) and (J), prepared in Example 4, (Figure 64).

## EXAMPLE 7

### **Competitive Inhibition of The Binding of HFE7A to Fas**

The expression products of Example 4 were evaluated for their ability to competitively inhibit the binding of HFE7A to the human Fas fusion protein, by a similar method to that described in Reference Example 19, but wherein DMEM was used as a diluent.

It was verified that each of the expression products of supernatants (B), (C), (D), (E), (F), (G), (H), (I) and (J) of Example 4 above specifically inhibited the binding of HFE7A prepared from a mouse hybridoma to the human Fas fusion protein (Figure 65).

**EXAMPLE 8****Apoptosis-Inducing Activity**

The apoptosis-inducing activity of each of the expression products in the culture supernatant fluids obtained in Example 4 was evaluated by the method described in Reference Example 20.

Each of the expression products of the culture supernatant fluids (B), (C), (D), (E), (F), (G), (H), (I) and (J), obtained in Example 4, were demonstrated to induce apoptosis in T cells of the lymphoma cell line expressing human Fas antigen (Figure 66).

**EXAMPLE 9****Construction of Expression Vector of Heavy Chain of Humanized HFE7A**

DNA was prepared encoding a humanized heavy chain, in which the CDR's of the HFE7A heavy chain, but not the FR, were grafted into the human antibody 8E10 heavy chain (hereinafter referred to as "HHH type humanized heavy chain"). The DNA and an expression vector plasmid having such DNA was prepared as follows:

**1) Synthesis of primer**

Synthesis of DNA (SEQ ID No. 156 of the Sequence Listing) encoding HHH type humanized heavy chain (SEQ ID No. 157 of the Sequence Listing) was carried out by PCR. The protein encoded by the DNA comprises a variable region (wherein each of CDR's of HFE7A heavy chain is grafted into the human monoclonal antibody 8E10 at the position corresponding thereto) and a constant region

of human Ig heavy chain ( $\gamma$ -chain). For PCR, the following four oligonucleotide primers were synthesized in addition to the above mentioned 7AH1P (SEQ ID No. 76 of the Sequence Listing) and H5- (SEQ ID No. 103 of the Sequence Listing):

5'- GATGCAGTGG GTACGACAGG CCCCTGGAC -3'

(HFR1F : SEQ ID No. 158 of the Sequence Listing);

5'- GTCCAGGGGC CTGTCGTACC CACTGCATC -3'

(HFR1B : SEQ ID No. 159 of the Sequence Listing);

5'- CAAGGGCCGG GTCACAATCA CTCGAGACAC ATC -3'

(HFR2F : SEQ ID No. 160 of the Sequence Listing); and

5'- GATGTGTCTC GAGTGATTGT GACCCGGCCC TTG -3'

(HFR2B : SEQ ID No. 161 of the Sequence Listing).

## 2) Preparation of DNA encoding HHH type humanized heavy chain

The HHH-DNA fragment (SEQ ID No. 156 of the Sequence Listing), encoding the amino acid sequence of SEQ ID No. 157 of the Sequence Listing, was prepared by PCR, then inserted into a plasmid and cloned into *E. coli*. The plasmid pgHPDHV3, constructed in Reference Example 23, was used as a template for PCR.

The heavy chain encoded by the plasmid pgHPDHV3 has the same amino acid sequence as the FR's of the 8E10 heavy chain, except that the 38th amino acid (Arg), the 66th amino acid (Arg), the 67th amino acid (Val), the 69th amino acid (Ile) and the 71st amino acid (Arg) are, instead, Lys, Lys, Ala, Leu and Val, respectively (hereinafter, the amino acid numbering is defined in accordance with Kabat et al., *supra*). Other amino acids in the FR and amino acids in the constant region are not replaced.

The CDR's in the amino acid sequence of the heavy chain encoded by pgHPDHV3 are the same as the CDR's in murine HFE7A. Accordingly, in the following steps, pgHPDHV3 (encoding a heavy

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chain amino acid sequence) was modified so that amino acids at the above mentioned positions were the same as those in the FR's of the 8E10 heavy chain.

a) First stage PCR

The outline of the first stage PCR for the preparation of HHHV-DNA is shown in Figure 67.

The HHHV1-DNA fragment, encoding a secretion signal sequence, the FRH<sub>1</sub> region, the CDRH<sub>1</sub> region, and a portion of the FRH<sub>2</sub> region altered to contain a Hind III restriction enzyme cleavage site at the 5'-end, was prepared as follows.

Composition of the PCR solution:

plasmid pgHPDV3 DNA, 200 ng;  
primer 7AH1P, 80 pmol;  
primer HFR1B, 80 pmol;  
dNTP cocktail, 20 µl;  
10 x Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water was added so that final volume of composition was 200 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, and then a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After this procedure, the reaction solution was heated at 72°C for 10 minutes.

The HHHV2-DNA fragment, encoding a portion of the FRH<sub>2</sub> region, the CDRH<sub>2</sub> region, and a portion of the FRH<sub>3</sub> region, was prepared as follows.

Composition of the PCR reaction solution:

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plasmid pgHPDV3 DNA, 200 ng;  
primer HFR1F, 80 pmol;  
primer HFR2B, 80 pmol;  
dNTP cocktail, 20 µl;  
10 x Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, and then a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes was repeated 30 times. After this procedure, the reaction solution was heated at 72°C for 10 minutes.

The HHV3-DNA fragment, encoding a portion of the CDRH<sub>2</sub> region and a portion of the FRH<sub>3</sub> region, was prepared as follows.

Composition of the PCR reaction solution:

plasmid pgHPDV3 DNA, 200 ng;  
primer HFR2F, 80 pmol;  
primer H5-, 80 pmol;  
dNTP cocktail, 20 µl;  
10 x Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, and then a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes was repeated 30 times. After completion of this procedure, the reaction solution was heated at 72°C for 10 minutes.

After extraction with phenol and precipitation with ethanol,

the resulting DNA precipitate was subjected to 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the acrylamide gel was stained with a 1 µg/ml of ethidium bromide to allow detection of DNA band under UV light. The DNA bands corresponding to HHHV1-DNA, HHHV2-DNA and HHHV3-DNA were excised, using a razor blade, and DNA was eluted from the gel using Centricon and Centrifiruter. The eluted DNA was concentrated first by centrifugation at 7,500 x g, then precipitated with ethanol, and finally dissolved in 50 µl of distilled water.

b) Second stage PCR

The outline of the second stage PCR for the production of HHHV-DNA is shown in Figure 68.

HHHV-DNA, in which the HHHV1-DNA, HHHV2-DNA and HHHV3-DNA fragments described above were fused, was prepared as follows.

Composition of the PCR reaction:

HHHV1-DNA solution prepared in the first stage PCR, 10 µl;  
HHHV2-DNA solution prepared in the first stage PCR, 10 µl;  
HHHV3-DNA solution prepared in the first stage PCR, 10 µl;  
primer 7AH1P, 80 pmol;  
primer H5-, 80 pmol;  
dNTP cocktail, 20 µl;  
10 x Pfu buffer, 20 µl;  
Pfu DNA polymerase, 10 units; and  
redistilled water to a final volume of 200 µl.

PCR was conducted as follows. The solution was first heated at 94°C for 2 minutes, and then a cycle of heating to 94°C for 1 minute, 55°C for 1 minute and 72°C for 2 minutes, was repeated 30 times. After this procedure, the reaction solution was heated at 72°C for 10 minutes.

After extraction with phenol and precipitation with ethanol, the resulting DNA precipitate was subjected to 5% w/v polyacrylamide gel electrophoresis. After electrophoresis, the acrylamide gel was stained with a 1 µg/ml of ethidium bromide to allow detection of DNA band under UV light. The DNA band corresponding to HHV-DNA was excised, using a razor blade, and DNA was eluted from the gel using Centricon and Centrifiruter. The eluted DNA was concentrated first by centrifugation at 7,500 × g, precipitated with ethanol, then dissolved in 50 µl of distilled water.

c) Construction of plasmid pgHSHHH1

The outline of the method of construction of the plasmid pgHSHHH1 carrying the HHV-DNA fragment is shown in Figure 69.

The HHV-DNA fragment, obtained above, was further purified by extraction with phenol and precipitation with ethanol, after which it was digested with the restriction enzymes Hind III and SacI. One µg of plasmid pgHPDV3 was digested with the restriction enzymes Hind III and SacI, and then dephosphorylated with CIP. The resulting dephosphorylated plasmid pgHPDV3 DNA and the digested HHV-DNA fragment were ligated using a DNA Ligation Kit (Version 2.0, Takara Shuzo Co., Ltd.). The ligated DNA was then used to transform *E. coli* JM 109. The cells were plated onto LB agar medium containing final concentrations of 50 µg/ml of chloramphenicol, and were cultured at 37°C.

Transformants thus obtained were cultured in liquid LB medium containing 50 µg/ml of chloramphenicol, and the plasmid DNA was extracted from the resulting culture by the alkaline-SDS method [Sambrook et al., *supra*]. The resulting, extracted plasmid DNA was digested with the restriction enzymes Hind III and SacI, and a clone carrying the HHV-DNA fragment was then

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selected by 1% w/v agarose gel electrophoresis and ethidium bromide staining.

Plasmid pgHSHHH1 carrying DNA encoding a fusion polypeptide of the variable region of the HHH type humanized heavy chain and the constant region of human IgG1  $\gamma$  chain was obtained accordingly.

### 3) Verification of the nucleotide sequences

In order to verify whether the DNA inserts of plasmid pgHSHHH1 obtained in the above 2) had the desired nucleotide sequence, its sequence was determined. The following oligonucleotides were used to determine the sequence as a primer for sequencing: the commercially available oligonucleotide primer RV (Takara Shuzo. Co. Ltd.); the above-mentioned 7AH1P (SEQ ID No. 76 of the Sequence Listing), HFR1F (SEQ ID No. 158 of the Sequence Listing), HFR1B (SEQ ID No. 159 of the Sequence Listing), HFR2F (SEQ ID No. 160 of the Sequence Listing), HFR2B (SEQ ID No. 161 of the Sequence Listing), H5- (SEQ ID No. 103 of the Sequence Listing) and the following four newly synthesized primers:

5'- CTACAATCAA AAGTTCAAGG -3'  
(SACF; SEQ ID No. 162);  
5'- GACTATAGTA ACAACTGGTA C -3'  
(APAF; SEQ ID No. 163);  
5'- GTACCAGTTG TTACTATAGT C -3'  
(SACB; SEQ ID No. 164); and  
5'- GCAGCCCAGG GCCGCTGTGC -3'  
(APAB; SEQ ID No. 165).

The positions to which each primer binds are shown in Figure 70.

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As a result, it was established that the plasmid pgHSHHH1 had the nucleotide sequences of SEQ ID No. 156 of the Sequence Listing encoding the polypeptide of SEQ ID No. 157 of the Sequence Listing. The transformant *E. coli* pgHSHHH1 SANK 72198, harboring plasmid pgHSHHH1, was deposited in the Kogyo Gijutsuin Seimei-Kogaku Kogyo Gijutsu Kenkyujo on September 18, 1998, in accordance with the Budapest Treaty, and was accorded the accession number FERM BP-6510.

#### 4) Construction of expression plasmid

The expression plasmid vector pSRHHH, carrying the DNA encoding HHH type humanized heavy chain polypeptide (SEQ ID No. 156 of the Sequence Listing), was constructed using the plasmid pgHSHHH1 obtained in 3) above. The procedure for construction of the plasmid pSRHHH is outlined in Figure 71.

One µg of plasmid pSRgPDH DNA (c.f. Reference Example 23) was digested with the restriction enzymes Hind III and SacI, and then dephosphorylated using CIP. The resulting dephosphorylated pSRgPDH DNA (100 ng) was ligated with 10 µg of the pgHSHHH1 DNA fragment which had also been digested with Hind III and SacI, using a DNA Ligation Kit (Version 2.0, Takara Shuzo Co., Ltd.). The ligation mix was then used to transform *E. coli* JM109, cells of which were then plated on LB agar plates containing 50 µg/ml of ampicillin, and cultured at 37°C.

All resulting transformants were cultured in liquid LB medium containing 50 µg/ml of ampicillin, and the plasmid DNA was extracted from the culture by the alkaline-SDS method [Sambrook et al., *supra*]. After the plasmid DNA was digested with Hind III and SacI, the presence or absence of the desired insert fragment was confirmed by 1% w/v agarose gel electrophoresis, stained with ethidium bromide. The plasmid pSRHHH, which contains DNA

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encoding the HHH type humanized heavy chain was inserted downstream of the SRα promoter in the correct orientation was, thus, obtained.

#### EXAMPLE 10

##### Construction of Expression Vector of light Chain of Humanized HFE7A

PLPDHH75, prepared in Reference Example 21, was used as an expression plasmid vector carrying DNA (SEQ ID No. 107 of the Sequence Listing) encoding a humanized light chain (SEQ ID No. 106 of the Sequence Listing). In this construction, CDR's of HFE7A light chain are grafted into the human antibody 8E10'CL light chain, but no FR's.

#### EXAMPLE 11

##### Expression in COS-1 Cells

COS-1 cells were grown to semi-confluence in α(+)MEM containing 10% v/v FCS (Moregate) in a culture flask (culture area: 225 cm<sup>2</sup>). The medium was then discarded, and 3 ml of trypsin-EDTA solution (Sigma) was added to the flask, which was then incubated at 37 °C for 3 minutes to detach the cells from the flask. The detached cells were then harvested by centrifugation at 800 r.p.m. for 2 minutes, and washed twice with PBS(-). The COS-1 cells were then suspended so that cell density was 1 × 10<sup>8</sup> cells/ml in PBS(-) buffer.

In parallel, 10 µg of humanized HFE7A heavy chain expression plasmid DNA were mixed with 10 µg of humanized HFE7A

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light chain expression plasmid DNA, each of which was prepared by the alkaline-SDS method and cesium chloride density gradient centrifugation [Sambrook et al., *supra*]. Ethanol was added to the mixture to precipitate the DNA and the precipitate was suspended in 20 µl of PBS(-). The resulting plasmid suspension (20 µl) was mixed with 20 µl of the previously prepared COS-1 cell suspension ( $2 \times 10^6$  cells) and the mixture was transferred to an FCT-13 chamber (Shimadzu Seisakusho, K. K.) having electrodes set 2 mm apart, and the chamber was then loaded into gene transfection apparatus GTE-1 (Shimadzu Seisakusho, K. K.). The desired plasmid DNA was transformed into the COS-1 cells by applying pulses of 600 V, 50 µF twice with a one second interval.

After this time, the solution of cell-DNA mixture in the chamber was suspended in 5 ml of α(+)MEM containing 10% v/v FCS and transferred to a culture flask (culture area 25 cm<sup>2</sup>). After incubating under 5% v/v CO<sub>2</sub> at 37°C for 72 hours, the culture supernatant was recovered.

Using the above method, COS-1 cells were variously transfected with each of the following plasmid combinations, and the supernatant thereof was recovered:

[A] : no plasmid DNA

[B] ; cotransfection with pSRgPDH and pSRPDHH

[C] ; cotransfection with pSRHHH and pSRPDHH

#### EXAMPLE 12

##### Quantification of Expressed Products by ELISA Method

The expression of humanized antibody in the culture supernatant prepared in Example 11 was verified and the quantification of the expressed products was performed in

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accordance with the method described in Reference Example 17. As a result, it was verified that each of the expressed products of culture supernatants prepared in Example 11 using [B] and [C] was specifically detected by the anti-human IgG antibody.

### EXAMPLE 13

#### Determination of Binding Activity to Fas

The culture supernatants obtained in Example 11 were adjusted to final concentrations of 100 ng/ml of the desired humanized antibody product, in  $\alpha(+)$  MEM containing 10% v/v FCS. Concentrations were calculated by the method described in Reference Example 17. When the concentration of the desired product was less than 100 ng/ml, the culture supernatant was first concentrated by Centriprep-10 (Amicon, Co. Ltd.). That is, 5 ml of the culture supernatant was transferred into the Centriprep-10 followed by concentrating by centrifugation at 3,000  $\times$  g, and again the concentration of the desired product was calculated by the method described in Reference Example 17.

Each of the resulting solutions, adjusted to 100 ng/ml, was then diluted by serial 2-fold dilution with  $\alpha(+)$  MEM containing 10% v/v FCS. The binding activity to human Fas fusion protein was determined by the ELISA method described in Reference Example 18.

The binding activity to the human Fas fusion protein was verified for the supernatants prepared in Example 11 using [B] and [C] and is shown in Figure 72.

**EXAMPLE 14****Competitive Inhibition of the Binding of HFE7A to Fas**

The competitive inhibitory activity of each of the expressed products prepared in Example 11 against the human Fas fusion protein, along with that of HFE7A, was determined by a similar method to that described in Reference Example 26.

It was verified that each of the expression products prepared in Example 11 specifically inhibited the binding of HFE7A prepared from a mouse hybridoma to the human Fas fusion protein (Figure 73).

**EXAMPLE 15****Apoptosis-Inducing Activity**

The culture supernatants obtained in Example 11 were adjusted so that the final concentration of antibody was 100 ng/ml, by a similar method to that described in Reference Example 22 (RPMI 1640 medium containing 10% v/v FCS was used as diluent, as necessary). Each of the solutions of the expression products was then diluted in serial 2-fold dilution with RPMI 1640 containing 10% v/v FCS. The cytotoxic activity of each sample to WR19L12a cells was determined by the method described in Reference Example 20.

As expected, each of the expression products of the culture supernatant obtained in Example 11 using [B] and [C] were demonstrated to induce apoptosis in T cells of this lymphoma cell line expressing human Fas antigen, in a similar manner to HFE7A prepared from the hybridoma cultures.

## SEQUENCE LISTING

<110> Serizawa, Nobufusa  
 Haruyama, Hideyuki  
 Nakahara, Kaori  
 Tamaki, Ikuko  
 Takahashi, Tohru

<120> Anti-Fas Antibodies

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1 5

<210> 7  
<211> 9  
<212> PRT  
<213> Mus musculus

<400> 7  
Gln Gln Ser Asn Glu Asp Pro Arg Thr  
1 5

<210> 8  
<211> 1392  
<212> DNA  
<213> Mus musculus

<220>  
<221> CDS  
<222> (1)..(1392)

<220>  
<221> mat peptide  
<222> (58)..(1392)

&lt;220&gt;

&lt;221&gt; sig peptide

&lt;222&gt; (1)..(57)

&lt;400&gt; 8

atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gct aca ggt 48  
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
     -15                       -10                       -5

gtc cat tct cag gtc caa ctg cag cag cct ggg gct gag ctt gtg aag 96  
 Val His Ser Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys  
     -1      1                      5                       10

cct ggg gct tca gtg aag ctg tcc tgc aag gct tct ggc tac acc ttc 144  
 Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
     15                       20                       25

acc agc tac tgg atg cag tgg gta aaa cag agg cct gga cag ggc ctt 192  
 Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Arg Pro Gly Gln Gly Leu  
     30                       35                       40                       45

gag tgg atc gga gag att gat cct tct gat agc tat act aac tac aat 240  
 Glu Trp Ile Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn  
     50                       55                       60

caa aag ttc aag ggc aag gcc aca ttg act gta gac aca tcc tcc agc 288  
 Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser  
     65                       70                       75

aca gcc tac atg cag ctc agc agc ctg aca tct gag gac tct gcg gtc 336  
 Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val  
     80                       85                       90

tat tac tgt gca aga aat agg gac tat agt aac aac tgg tac ttc gat 384  
 Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp  
     95                       100                       105

gtc tgg ggc aca ggg acc acg gtc acc gtc tcc tca gcc aaa acg aca 432  
 Val Trp Gly Thr Gly Thr Val Thr Val Ser Ser Ala Lys Thr Thr  
     110                       115                       120                       125

ccc cca tct gtc tat cca ctg gcc cct gga tct gct gcc caa act aac 480  
 Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn  
     130                       135                       140

tcc atg gtg acc ctg gga tgc ctg gtc aag ggc tat ttc cct gag cca 528  
 Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro  
     145                       150                       155

gtg aca gtg acc tgg aac tct gga tcc ctg tcc agc ggt gtg cac acc 576  
 Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr  
     160                       165                       170

tcc cca gct gtc ctg cag tct gac ctc tac act ctg agc agc tca gtg 624

Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr	Leu Ser Ser Ser Val		
175	180	185	
act gtc ccc tcc agc acc tgg ccc agc cag acc	gtc acc tgc aac gtt	672	
Thr Val Pro Ser Ser Thr Trp Pro Ser Gln	Thr Val Thr Cys Asn Val		
190	195	200	205
gcc cac ccg gcc agc agc acc aag gtg gac aag	aaa att gtg ccc agg	720	
Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg			
210	215	220	
gat tgt ggt tgt aag cct tgc ata tgt aca gtc cca gaa	gta tca tct	768	
Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser			
225	230	235	
gtc ttc atc ttc ccc cca aag ccc aag gat gtg ctc acc att act ctg		816	
Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu			
240	245	250	
act cct aag gtc acg tgt gtt gtg gta gac atc agc aag gat gat ccc		864	
Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro			
255	260	265	
gag gtc cag ttc agc tgg ttt gta gat gat gtg gag gtg cac aca gct		912	
Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala			
270	275	280	285
cag acg caa ccc cg <sup>g</sup> gag gag cag ttc aac agc act ttc cgc tca gtc		960	
Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val			
290	295	300	
agt gaa ctt ccc atc atg cac cag aac tgg ctc aat ggc aag gag ttc		1008	
Ser Glu Leu Pro Ile Met His Gln Asn Trp Leu Asn Gly Lys Glu Phe			
305	310	315	
aaa tgc agg gtc aac agt gca gct ttc cct gcc ccc atc gag aaa acc		1056	
Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr			
320	325	330	
atc tcc aaa acc aaa ggc aga ccg aag gct cca cag gtg tac acc att		1104	
Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile			
335	340	345	
cca cct ccc aag gag cag atg gcc aag gat aaa gtc agt ctg acc tgc		1152	
Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys			
350	355	360	365
atg ata aca gac ttc ttc cct gaa gac att act gtg gag tgg cag tgg		1200	
Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp			
370	375	380	

aat ggg cag cca gcg gag aac tac aag aac act cag ccc atc atg aac		1248
Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asn		
385	390	395
acg aat ggc tct tac ttc gtc tac agc aag ctc aat gtg cag aag agc		1296
Thr Asn Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser		
400	405	410
aac tgg gag gca gga aat act ttc acc tgc tct gtg tta cat gag ggc		1344
Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly		
415	420	425
ctg cac aac cac cat act gag aag agc ctc tcc cac tct cct ggt aaa		1392
Leu His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys		
430	435	440

<210> 9  
<211> 464  
<212> PRT  
<213> Mus musculus

<400> 9		
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly		
-15	-10	-5
Val His Ser Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys		
-1 1	5	10
Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe		
15	20	25
Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Arg Pro Gly Gln Gly Leu		
30	35	40
Glu Trp Ile Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn		
50	55	60
Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser		
65	70	75
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val		
80	85	90
Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp		
95	100	105
Val Trp Gly Thr Gly Thr Thr Val Thr Val Ser Ser Ala Lys Thr Thr		
110	115	120
Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn		
130	135	140

Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro  
 145 150 155

Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr  
 160 165 170

Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val  
 175 180 185

Thr Val Pro Ser Ser Thr Trp Pro Ser Gln Thr Val Thr Cys Asn Val  
 190 195 200 205

Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg  
 210 215 220

Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser  
 225 230 235

Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu  
 240 245 250

Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro  
 255 260 265

Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala  
 270 275 280 285

Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val  
 290 295 300

Ser Glu Leu Pro Ile Met His Gln Asn Trp Leu Asn Gly Lys Glu Phe  
 305 310 315

Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr  
 320 325 330

Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile  
 335 340 345

Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys  
 350 355 360 365

Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp  
 370 375 380

Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asn  
 385 390 395

Thr Asn Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser  
 400 405 410

Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly  
 415 420 425

Leu His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys  
 430                    435                    440                    445

<210> 10  
 <211> 714  
 <212> DNA  
 <213> Mus musculus

<220>  
 <221> CDS  
 <222> (1)..(714)

<220>  
 <221> mat peptide  
 <222> (61)..(714)

<220>  
 <221> sig peptide  
 <222> (1)..(60)

<400> 10  
 atg gag aca gac aca atc ctg cta tgg gtg atg atg ctc tgg att cca      48  
 Met Glu Thr Asp Thr Ile Leu Leu Trp Val Met Met Leu Trp Ile Pro  
 -20                    -15                    -10                    -5

ggc tcc act ggt gac att gtg ctg acc caa tct cca gct tct ttg gct      96  
 Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala  
 -1      1            5                    10

gtg tct cta ggg cag agg gcc acc atc tcc tgc aag gcc agc caa agt      144  
 Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser  
 15                    20                    25

gtt gat tat gat ggt gat agt tat atg aac tgg tac caa cag aaa cca      192  
 Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro  
 30                    35                    40

gga cag cca ccc aaa ctc ctc atc tat gct gca tcc aat cta gaa tct      240  
 Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
 45                    50                    55                    60

ggg atc cca gcc agg ttt agt ggc agt ggg tct ggg aca gac ttc acc      288  
 Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr  
 65                    70                    75

ctc aac atc cat cct gtg gag gag gag gat gct gca acc tat tac tgt      336  
 Leu Asn Ile His Pro Val Glu Glu Asp Ala Ala Thr Tyr Tyr Cys  
 80                    85                    90

cag caa agt aat gag gat cct cggt acg ttc ggt gga ggc acc aag ctg      384  
 Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gly Thr Lys Leu  
 95                    100                    105

gaa atc aaa cgg gct gat gct gca cca act gta tcc atc ttc cca cca 432  
 Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro  
 110 115 120

tcc agt gag cag tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg 480  
 Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu  
 125 130 135 140

aac aac ttc tac ccc aaa gac atc aat gtc aag tgg aag att gat ggc 528  
 Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly  
 145 150 155

agt gaa cga caa aat ggc gtc ctg aac agt tgg act gat cag gac agc 576  
 Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser  
 160 165 170

aaa gac agc acc tac agc atg agc agc acc ctc acg ttg acc aag gac 624  
 Lys Asp Ser Thr Tyr Ser Met Ser Thr Leu Thr Leu Thr Lys Asp  
 175 180 185

gag tat gaa cga cat aac agc tat acc tgt gag gcc act cac aag aca 672  
 Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr  
 190 195 200

tca act tca ccc att gtc aag agc ttc aac agg aat gag tgt 714  
 Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys  
 205 210 215

<210> 11  
 <211> 238  
 <212> PRT  
 <213> Mus musculus

<400> 11  
 Met Glu Thr Asp Thr Ile Leu Leu Trp Val Met Met Leu Trp Ile Pro  
 -20 -15 -10 -5

Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala  
 -1 1 5 10

Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser  
 15 20 25

Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro  
 30 35 40

Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
 45 50 55 60

Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
 65 70 75

Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys  
 80 85 90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gly Thr Lys Leu  
 95 100 105

Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro  
 110 115 120

Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu  
 125 130 135 140

Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly  
 145 150 155

Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser  
 160 165 170

Lys Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp  
 175 180 185

Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr  
 190 195 200

Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys  
 205 210 215

<210> 12

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to  
 amplify a DNA encoding the extracellular region of  
 human Fas antigen

<400> 12

gggaaattcc agtacggagt tggggaaagct cttt

34

<210> 13

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to  
 amplify a DNA encoding the extracellular region of  
 human Fas antigen

<400> 13

gtttcttctg cctctgtcac caagtttagat ctgga

35

<210> 14  
<211> 35  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a DNA encoding the extracellular region of mouse IL-3 receptor

<400> 14 tccagatcta acttggtgac agaggcagaa gaaac 35

<210> 15  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a DNA encoding the extracellular region of mouse IL-3 receptor

<400> 15 ccctcttagac gcgtcacgtg ggcacatcac 28

<210> 16  
<211> 11  
<212> PRT  
<213> Mus musculus

<220>  
<221> Unsure  
<222> 2  
<223> Unidentified amino acid

<400> 16  
Gln Xaa Gln Leu Gln Gln Pro Gly Ala Glu Leu  
1 5 10

<210> 17  
<211> 22  
<212> PRT  
<213> Mus musculus

<400> 17  
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly  
1 5 10 15

Gln Arg Ala Thr Ile Ser  
20

<210> 18  
<211> 19  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a DNA encoding mouse immunoglobulin heavy  
chain gamma 1 subtype 2b

<400> 18  
gacctcacca tggatgga

19

<210> 19  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a DNA encoding mouse immunoglobulin heavy  
chain gamma 1 subtype 2b

<400> 19  
tttaccagga gagtggaga

20

<210> 20  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a DNA encoding mouse immunoglobulin light  
chain kappa subtype 3

<400> 20  
aagaagcatc ctctcatcta

20

<210> 21  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a DNA encoding mouse immunoglobulin light

chain kappa subtype 3

<400> 21  
acactcattc ctgttgaagc

20

<210> 22  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Adaptor primer  
to subclone a cDNA encoding the heavy chain of  
anti-human Fas antibody HFE7A

<400> 22  
gggaaattcg acctcaccat gggatgga

28

<210> 23  
<211> 32  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Adaptor primer  
to subclone a cDNA encoding the heavy chain of  
anti-human Fas antibody HFE7A

<400> 23  
gggtctagac tatttaccag gagagtggga ga

32

<210> 24  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Adaptor primer  
to subclone a cDNA encoding the light chain of  
anti-human Fas antibody HFE7A

<400> 24  
gggaaattca agaagcatcc tctcatcta

29

<210> 25  
<211> 37  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Adaptor primer  
to subclone a cDNA encoding the light chain of  
anti-human Fas antibody HFE7A

<400> 25

ggggcgccg cttactaaca ctcattcctg ttgaagc

37

<210> 26

<211> 19

<212> PRT

<213> Homo sapiens

<400> 26

Arg Leu Ser Ser Lys Ser Val Asn Ala Gln Val Thr Asp Ile Asn Ser  
1 5 10 15

Lys Gly Leu

<210> 27

<211> 19

<212> PRT

<213> Homo sapiens

<400> 27

Val Thr Asp Ile Asn Ser Lys Gly Leu Glu Leu Arg Lys Thr Val Thr  
1 5 10 15

Thr Val Glu

<210> 28

<211> 20

<212> PRT

<213> Homo sapiens

<400> 28

Glu Leu Arg Lys Thr Val Thr Val Glu Thr Gln Asn Leu Glu Gly  
1 5 10 15

Leu His His Asp  
20

<210> 29

<211> 20

<212> PRT

<213> Homo sapiens

<400> 29

Thr Gln Asn Leu Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys  
1 5 10 15

Pro Cys Pro Pro  
20

<210> 30  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 30  
Gly Gln Phe Cys His Lys Pro Cys Pro Pro Gly Glu Arg Lys Ala Arg  
1 5 10 15

Asp Cys Thr Val  
20

<210> 31  
<211> 21  
<212> PRT  
<213> Homo sapiens

<400> 31  
Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro Asp  
1 5 10 15

Cys Val Pro Cys Gln  
20

<210> 32  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 32  
Asn Gly Asp Glu Pro Asp Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr  
1 5 10 15

Thr Asp Lys Ala  
20

<210> 33  
<211> 19  
<212> PRT  
<213> Homo sapiens

<400> 33  
Glu Gly Lys Glu Tyr Thr Asp Lys Ala His Phe Ser Ser Lys Cys Arg  
1 5 10 15

Arg Cys Arg

<210> 34  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 34  
His Phe Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly His  
1 5 10 15

Gly Leu Glu Val  
20

<210> 35  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 35  
Leu Cys Asp Glu Gly His Gly Leu Glu Val Glu Ile Asn Cys Thr Arg  
1 5 10 15

Thr Gln Asn Thr  
20

<210> 36  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 36  
Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys Cys Arg Cys Lys Pro  
1 5 10 15

Asn Phe Phe Cys  
20

<210> 37  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 37  
Lys Cys Arg Cys Lys Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu  
1 5 10 15

His Cys Asp Pro  
20

<210> 38  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 38  
Asn Ser Thr Val Cys Glu His Cys Asp Pro Cys Thr Lys Cys Glu His  
1 5 10 15  
Gly Ile Ile Lys  
20

<210> 39  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 39  
Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr Ser  
1 5 10 15  
Asn Thr Lys Cys  
20

<210> 40  
<211> 18  
<212> PRT  
<213> Homo sapiens

<400> 40  
Glu Cys Thr Leu Thr Ser Asn Thr Lys Cys Lys Glu Glu Gly Ser Arg  
1 5 10 15

Ser Asn

<210> 41  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 41  
Ser Ser Gly Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala  
1 5 10 15

Phe Asn Val Glu  
20

<210> 42

<211> 10  
<212> PRT  
<213> Homo sapiens

<400> 42  
His Gly Leu Glu Val Glu Ile Asn Cys Thr  
1 5 10

<210> 43  
<211> 10  
<212> PRT  
<213> Homo sapiens

<400> 43  
Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr  
1 5 10

<210> 44  
<211> 10  
<212> PRT  
<213> Homo sapiens

<400> 44  
Lys Cys Arg Cys Lys Pro Asn Phe Phe Cys  
1 5 10

<210> 45  
<211> 14  
<212> PRT  
<213> Homo sapiens

<400> 45  
Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His Cys Asp  
1 5 10

<210> 46  
<211> 10  
<212> PRT  
<213> Homo sapiens

<400> 46  
Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn  
1 5 10

<210> 47  
<211> 34  
<212> DNA  
<213> Homo sapiens

<400> 47  
gcgaattctg ctttactga tcagagtttc ctca

34

<210> 48  
<211> 32  
<212> DNA  
<213> Homo sapiens

<400> 48  
gctcttagatg aggtgaaaga tgagctggag ga

32

<210> 49  
<211> 768  
<212> DNA  
<213> Artificial Sequence

<220>  
<221> CDS  
<222> (40)..(753)

<220>  
<221> mat peptide  
<222> (100)..(753)

<220>  
<221> sig peptide  
<222> (40)..(99)

<220>  
<223> Description of Artificial Sequence: Designed DNA  
encoding the light chain of humanized anti-human  
Fas antibody

<400> 49  
cccaagctta agaaggcatcc tctcatctag ttctcagag atg gag aca gac aca 54  
Met Glu Thr Asp Thr  
-20

atc ctg cta tgg gtg ctg ctc tgg gtt cca ggc tcc act ggt gac 102  
Ile Leu Leu Trp Val Leu Leu Trp Val Pro Gly Ser Thr Gly Asp  
-15 -10 -5 -1 1

att gtg ctc acc caa tct cca ggt act ttg tct ctg tct cca ggg gag 150  
Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu  
5 10 15

agg gcc acc ctc tcc tgc aag gcc agc caa agt gtt gat tat gat ggt 198  
Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp Gly  
20 25 30

```
<210> 50
<211> 238
<212> PRT
<213> Artificial Sequence
```

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: Designed light chain of humanized anti-Fas antibody

&lt;400&gt; 50

Met	Glu	Thr	Asp	Thr	Ile	Leu	Leu	Trp	Val	Leu	Leu	Leu	Trp	Val	Pro
-20					-15					-10					-5

Gly	Ser	Thr	Gly	Asp	Ile	Val	Leu	Thr	Gln	Ser	Pro	Gly	Thr	Leu	Ser
-1	1						5					10			

Leu	Ser	Pro	Gly	Glu	Arg	Ala	Thr	Leu	Ser	Cys	Lys	Ala	Ser	Gln	Ser
		15				20					25				

Val	Asp	Tyr	Asp	Gly	Asp	Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Pro
30						35					40				

Gly	Gln	Ala	Pro	Arg	Leu	Leu	Ile	Tyr	Ala	Ala	Ser	Asn	Leu	Glu	Ser
45					50				55				60		

Gly	Ile	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr		
	65						70				75				

Leu	Thr	Ile	Ser	Arg	Leu	Glu	Pro	Ala	Asp	Phe	Ala	Val	Tyr	Tyr	Cys
	80					85					90				

Gln	Gln	Ser	Asn	Glu	Asp	Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Arg	Leu
	95					100					105				

Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro
	110					115					120				

Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu
125					130				135			140			

Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn
	145							150				155			

Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser
	160						165				170				

Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala
	175					180				185					

Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly
	190					195				200					

Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys		
205					210				215						

&lt;210&gt; 51

&lt;211&gt; 768

<212> DNA  
<213> Artificial Sequence

<220>  
<221> CDS  
<222> (40) .. (753)

```
<220>
<221> mat peptide
<222> (100)..(753)
```

<220>  
<221> sig peptide  
<222> (40) .. (99)

<220>

<223> Description of Artificial Sequence: Designed DNA encoding the light chain of humanized anti-human Fas antibody

<400> 51  
cccaagctta agaaggatcc tctcatctag ttctcagag atg gag aca gac aca 54  
Met Glu Thr Asp Thr  
-20

atc ctg cta tgg gtg ctg ctg ctc tgg gtt cca ggc tcc act ggt gac 102  
Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Asp  
-15 -10 -5 -1 1

```

agg gcc acc ctc tcc tgc aag gcc agc caa agt gtt gat tat gat ggt 198
Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp Gly
          20           25           30

```

gat agt tat atg aac tgg tac caa cag aaa cca gga cag gca ccc aga 246  
 Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg  
                  35                 40                 45

ctc ctc atc tat gct gca tcc aat ctc gaa tct ggg atc cca gac agg 294  
 Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Ile Pro Asp Arg  
       50              55                     60              65

ttt agt ggc agt ggg tct ggg aca gac ttc acc ctc acc acc atc cat cct 342  
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile His Pro  
70 75 80

gtg gag gag gag gat gct gca acc tat tac tgt cag caa agt aat gag 390  
 Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu  
 85 90 95

gat cct cg <sup>g</sup> acg ttc ggt caa gg <sup>c</sup> acc agg ctg gaa atc aaa cg <sup>g</sup> act	438
Asp Pro Arg Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr	
100 105 110	
gtg gct gca cca tct gtc ttc atc ttc cc <sup>g</sup> cca tct gat gag cag ttg	486
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu	
115 120 125	
aaa tct gga act gg <sup>c</sup> tct gtt gtg tgc ctg ctg aat aac tt <sup>c</sup> tat ccc	534
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro	
130 135 140 145	
aga gag gg <sup>c</sup> aaa gta cag tgg aaa gtg gat aac gg <sup>c</sup> ctc caa tcg ggt	582
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly	
150 155 160	
aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc tac	630
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr	
165 170 175	
agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac	678
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His	
180 185 190	
aaa gtc tac gg <sup>c</sup> tgc gaa gtc acc cat cag gg <sup>c</sup> ctg agc tcg ccc gtc	726
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val	
195 200 205	
aca aag agc tt <sup>c</sup> aac agg gga gag tgt tagtaagaat tcggg	768
Thr Lys Ser Phe Asn Arg Gly Glu Cys	
210 215	

<210> 52  
<211> 238  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Designed light  
chain of humanized anti-Fas antibody

Met Glu Thr Asp Thr Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro			
-20 -15 -10 -5			
Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser			
-1 1 5 10			
Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser			
15 20 25			
Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro			
30 35 40			

Gly Gln Ala Pro Arg Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
 45                   50                   55                   60

Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr  
 65                   70                   75

Leu Thr Ile His Pro Val Glu Glu Asp Ala Ala Thr Tyr Tyr Cys  
 80                   85                   90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Arg Leu  
 95                   100                  105

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro  
 110                  115                  120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
 125                  130                  135                  140

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn  
 145                  150                  155

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
 160                  165                  170

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
 175                  180                  185

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
 190                  195                  200

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 205                  210                  215

<210> 53  
<211> 768  
<212> DNA  
<213> Artificial Sequence

<220>  
<221> CDS  
<222> (40)..(753)

<220>  
<221> mat peptide  
<222> (100)..(753)

<220>  
<221> sig peptide  
<222> (40)..(99)

<220>  
<223> Description of Artificial Sequence: Designed DNA  
encoding the light chain of humanized anti-human

## Fas antibody

<400> 53  
 cccaaagctta agaaggcatcc tctcatctag ttctcagag atg gag aca gac aca 54  
 Met Glu Thr Asp Thr  
 -20

atc ctg cta tgg gtg ctg ctg tgg gtt cca ggc tcc act ggt gac 102  
 Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Asp  
 -15 -10 -5 -1 1

att gtg ctc acc caa tct cca ggt act ttg tct ctg tct cca ggg gag 150  
 Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu  
 5 10 15

agg gcc acc ctc tcc tgc aag gcc agc caa agt gtt gat tat gat ggt 198  
 Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp Gly  
 20 25 30

gat agt tat atg aac tgg tac caa cag aaa cca gga cag cca ccc aaa 246  
 Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Pro Pro Lys  
 35 40 45

ctc ctc atc tat gct gca tcc aat ctc gaa tct ggg atc cca gac agg 294  
 Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Ile Pro Asp Arg  
 50 55 60 65

ttt agt ggc agt ggg tct ggg aca gac ttc acc ctc acc atc cat cct 342  
 Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile His Pro  
 70 75 80

gtg gag gag gag gat gct gca acc tat tac tgt cag caa agt aat gag 390  
 Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu  
 85 90 95

gat cct cggt acg ttc ggt caa ggc acc agg ctg gaa atc aaa cggt act 438  
 Asp Pro Arg Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr  
 100 105 110

gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag ttg 486  
 Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu  
 115 120 125

aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc 534  
 Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro  
 130 135 140 145

aga gag gcc aaa gta cag tgg aaa gtg gat aac gcc ctc caa tcg ggt 582  
 Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly  
 150 155 160

aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc tac 630  
 Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr  
 165 170 175

agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac 678  
 Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His  
 180 185 190

aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc 726  
 Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val  
 195 200 205

aca aag agc ttc aac agg gga gag tgt tagtaagaat tcggg 768  
 Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 210 215

<210> 54  
 <211> 238  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Designed light chain of humanized anti-Fas antibody

<400> 54  
 Met Glu Thr Asp Thr Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
 -20 -15 -10 -5

Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser  
 -1 1 5 10

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser  
 15 20 25

Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro  
 30 35 40

Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
 45 50 55 60

Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
 65 70 75

Leu Thr Ile His Pro Val Glu Glu Asp Ala Ala Thr Tyr Tyr Cys  
 80 85 90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Arg Leu  
 95 100 105

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro  
 110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
 125 130 135 140

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn  
           145                     150                     155  
 Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
           160                     165                     170  
 Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
           175                     180                     185  
 Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
           190                     195                     200  
 Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
           205                     210                     215

<210> 55  
 <211> 34  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: PCR primer to  
       amplify a fragment of DNA encoding the light  
       chain of humanized anti-Fas antibody  
  
 <400> 55  
 cccaaagctta agaaggcatcc tctcatcttag ttct                 34

<210> 56  
 <211> 44  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: PCR primer to  
       amplify a fragment of DNA encoding the light  
       chain of humanized anti-Fas antibody  
  
 <400> 56  
 gagagggtgg ccctctcccc tggagacaga gacaaagtac ctgg         44

<210> 57  
 <211> 44  
 <212> DNA  
 <213> Artificial Sequence  
  
 <220>  
 <223> Description of Artificial Sequence: PCR primer to  
       amplify a fragment of DNA encoding the light  
       chain of humanized anti-Fas antibody

<400> 57  
 ccaggtactt tgtctctgtc tccagggag agggccaccc tctc 44

<210> 58  
 <211> 44  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of humanized anti-Fas antibody

<400> 58  
 gattcgagat tggatgcagc atagatgagg agtctgggtg cctg 44

<210> 59  
 <211> 45  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of humanized anti-Fas antibody

<400> 59  
 gctgcattca atctcgaatc tgggatccca gacaggtta gtggc 45

<210> 60  
 <211> 52  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of humanized anti-Fas antibody

<400> 60  
 aaaatccgccc ggctccagac gagagatggt gagggtaag tctgtccca ac 52

<210> 61  
 <211> 58  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light

chain of humanized anti-Fas antibody

<400> 61  
ctcgctcgga gccggcggat tttgcagtct attactgtca gcaaagtaat gaggatcc 58

<210> 62  
<211> 55  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the light  
chain of humanized anti-Fas antibody

<400> 62  
tgaagacaga tggtgccagcc acagtccgtt tgatttccag cctgggtgcct tgacc 55

<210> 63  
<211> 55  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the light  
chain of humanized anti-Fas antibody

<400> 63  
ggtcaaggca ccaggctgga aatcaaacgg actgtggctg caccatctgt ctta 55

<210> 64  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the light  
chain of humanized anti-Fas antibody

<400> 64  
cccgaaattct tactaacact ctccccctgtt gaagctcttt gtgac 45

<210> 65  
<211> 55  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of humanized anti-Fas antibody

<400> 65  
tctgtccca acccaactgcc actaaacctg tctggatcc cagattcgag attgg 55

<210> 66  
<211> 55  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of humanized anti-Fas antibody

<400> 66  
gttagtgtgc agtgggtctg ggacagactt cacctctacc atccatcctg tggag 55

<210> 67  
<211> 55  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of humanized anti-Fas antibody

<400> 67  
atggtgtcagc cacagtccgt ttgatttcca gcctggtgcc ttgaccgaac gtccg 55

<210> 68  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for DNAs encoding the light chains of humanized anti-Fas antibodies

<400> 68  
cccaagctta agaagcatcc 20

<210> 69  
<211> 20  
<212> DNA  
<213> Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Sequencing  
primer for DNAs encoding the light chains of  
humanized anti-Fas antibodies

&lt;400&gt; 69

atctatgctg catccaatct

20

&lt;210&gt; 70

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Sequencing  
primer for DNAs encoding the light chains of  
humanized anti-Fas antibodies

&lt;400&gt; 70

gttgttgcc tgctgaataa

20

&lt;210&gt; 71

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Sequencing  
primer for DNAs encoding the light chains of  
humanized anti-Fas antibodies

&lt;400&gt; 71

cccgaaattct tactaacact

20

&lt;210&gt; 72

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Sequencing  
primer for DNAs encoding the light chains of  
humanized anti-Fas antibodies

&lt;400&gt; 72

ttattcagca ggcacacaaac

20

&lt;210&gt; 73

&lt;211&gt; 20

&lt;212&gt; DNA

### <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequencing primer for DNAs encoding the light chains of humanized anti-Fas antibodies

<400> 73

agattggatg cagcatagat

20

<210> 74

<211> 457

<212> DNA

### <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: DNA encoding the partial peptide of the heavy chain of a humanized anti-Fas antibody

<220>

<221> CDS

<222> (21) .. (455)

<220>

<221> mat peptide

<222> (78)..(455)

<220>

<221> sig peptide

<222> (21)..(77)

<400> 74

```

aagcttggct tgacctcacc atg gga tgg agc tgt atc atc ctc ttc ttg gta 53
          Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val
          -15                  -10

```

```

gca aca gct aca ggt gtc cac tct cag gtc caa ctg gtg cag tct ggg   101
Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu Val Gln Ser Gly
          -5           -1    1           5

```

```

gct gag gtc aag aag cct ggg gct tca gtg aag gtg tcc tgc aag gct      149
Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala
          10           15           20

```

tct ggc tac acc ttc acc agc tac tgg atg cag tgg gta aaa cag gcc 197  
 Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Ala  
 25 30 35 40

cct gga cag agg ctt gag tgg atg gga gag att gat cct tct gat agc 245  
 Pro Gly Gln Arg Leu Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser  
45 50 55

tat act aac tac aat caa aag ttc aag ggc aag gcc aca ttg act gta	293		
Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val			
60	65	70	
gac aca tcc gct agc aca gcc tac atg gag ctc agc agc ctg aga tct	341		
Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser			
75	80	85	
gag gac acg gcg gtc tat tac tgt gca aga aat agg gac tat agt aac	389		
Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn			
90	95	100	
aac tgg tac ttc gat gtc tgg ggc gaa ggg acc ctg gtc acc gtc tcc	437		
Asn Trp Tyr Phe Asp Val Trp Gly Glu Gly Thr Leu Val Thr Val Ser			
105	110	115	120
tca gcc tcc acc aag ggc cc	457		
Ser Ala Ser Thr Lys Gly			
125			

&lt;210&gt; 75

&lt;211&gt; 145

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: Designed partial peptide of the heavy chain of humanized anti-human Fas antibody

&lt;400&gt; 75

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
-15 -10 -5Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
-1 1 5 10Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
15 20 25Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Ala Pro Gly Gln Arg Leu  
30 35 40 45Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn  
50 55 60Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ala Ser  
65 70 75Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val  
80 85 90

Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp  
95 100 105

Val Trp Gly Glu Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
110 115 120 125

Gly

<210> 76

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of the DNA encoding variable  
region in the heavy chain of a humanized anti-Fas  
antibody

<400> 76

ggaaagcttg gcttgcaccc accatgggat ggagctgtat

40

<210> 77

<211> 48

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of the DNA encoding variable  
region in the heavy chain of a humanized anti-Fas  
antibody

<400> 77

tgaaggccca ggcttcttga cctcagcccc agactgcacc agttggac

48

<210> 78

<211> 36

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of the DNA encoding variable  
region in the heavy chain of a humanized anti-Fas  
antibody

<400> 78

tccactcaag cctctgtcca ggggcctgtt ttaccc

36

<210> 79

<211> 52

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding variable region in the heavy chain of a humanized anti-Fas antibody

<400> 79

gtctgggct gaggtcaaga agcctggggc ttcagtgaag gtgtcctgca ag

52

<210> 80

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding variable region in the heavy chain of a humanized anti-Fas antibody

<400> 80

caggccccctg gacagaggct tgagtggatg ggagagatt

39

<210> 81

<211> 50

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding variable region in the heavy chain of a humanized anti-Fas antibody

<400> 81

tcatatctca ggctgcttag ctccatgttag gctgtgctag cggatgtgtc

50

<210> 82

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding variable region in the heavy chain of a humanized anti-Fas antibody

<400> 82  
tggagctcag cagcctgaga tctgaggaca cggcggtcta ttac 44

<210> 83  
<211> 55  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding variable region in the heavy chain of a humanized anti-Fas antibody

<400> 83  
gatgggccat tggtgaggc tgaggagacg gtgaccaggg tcccttcgcc ccagt 55

<210> 84  
<211> 39  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding the constant region of human immunoglobulin G1 heavy chain

<400> 84  
ggaaagcttc cgccgtcaca tggcaccacc tctttgca 39

<210> 85  
<211> 35  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding the constant region of human immunoglobulin G1 heavy chain

<400> 85  
gctctgcaga gagaagattg ggagttactg gaatc 35

<210> 86  
<211> 35  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding the constant region of human immunoglobulin G1 heavy chain

<400> 86  
tctctgcaga gcccaaatct tgtgacaaaa ctcac

35

<210> 87  
<211> 39  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding the constant region of human immunoglobulin G1 heavy chain

<400> 87  
ggggaaattcg ggagcggggc ttgccggccg tcgcactca

39

<210> 88  
<211> 2077  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: Designed DNA encoding the heavy chain of a humanized anti-Fas antibody

<220>  
<221> sig peptide  
<222> (27)..(83)

<220>  
<221> intron  
<222> (741)..(1131)

<220>  
<221> intron  
<222> (1177)..(1294)

<220>  
<221> intron

<222> (1625) .. (1721)

<220>

<221> exon

<222> (27) .. (740)

<220>

<221> exon

<222> (1132) .. (1176)

<220>

<221> exon

<222> (1295) .. (1624)

<220>

<221> exon

<222> (1722) .. (2042)

<220>

<221> mat peptide

<222> (84) .. (740)

<220>

<221> mat peptide

<222> (1132) .. (1176)

<220>

<221> mat peptide

<222> (1295) .. (1624)

<220>

<221> mat peptide

<222> (1722) .. (2042)

<220>

<221> CDS

<222> (27) .. (740)

<220>

<221> CDS

<222> (1132) .. (1176)

<220>

<221> CDS

<222> (1295) .. (1624)

<220>

<221> CDS

<222> (1722) .. (2042)

<400> 88

ggcgaaagc ttggcttgac ctcacc atg gga tgg agc tgt atc atc ctc ttc 53

Met Gly Trp Ser Cys Ile Ile Leu Phe

-15

ttg gta gca aca gct aca ggt gtc cac tct cag gtc caa ctg gtg cag		101	
Leu Val Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu Val Gln			
-10	-5	5	
tct ggg gct gag gtc aag aag cct ggg gct tca gtg aag gtg tcc tgc		149	
Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys			
10	15	20	
aag gct tct ggc tac acc ttc acc agc tac tgg atg cag tgg gta aaa		197	
Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Met Gln Trp Val Lys			
25	30	35	
cag gcc cct gga cag agg ctt gag tgg atg gga gag att gat cct tct		245	
Gln Ala Pro Gly Gln Arg Leu Glu Trp Met Gly Glu Ile Asp Pro Ser			
40	45	50	
gat agc tat act aac tac aatcaa aag ttc aag ggc aag gcc aca ttg		293	
Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu			
55	60	65	70
act gta gac aca tcc gct agc aca gcc tac atg gag ctc agc agc ctg		341	
Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu			
75	80	85	
aga tct gag gac acg gcg gtc tat tac tgt gca aga aat agg gac tat		389	
Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr			
90	95	100	
agt aac aac tgg tac ttc gat gtc tgg ggc gaa ggg acc ctg gtc acc		437	
Ser Asn Asn Trp Tyr Phe Asp Val Trp Gly Glu Gly Thr Leu Val Thr			
105	110	115	
gtc tcc tca gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc		485	
Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro			
120	125	130	
tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc		533	
Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val			
135	140	145	150
aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc		581	
Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala			
155	160	165	
ctg acc agc ggc gtg cac acc ttc ccg gct gtc cta cag tcc tca gga		629	
Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly			
170	175	180	
ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc agc agc ttg ggc		677	
Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly			
185	190	195	
acc cag acc tac atc tgc aac gtg aat cac aag ccc agc aac acc aag		725	

Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys  
200 205 210

gtg gac aag aga gtt ggtgagagggc cagcacaggg agggaggggtg tctgctggaa 780  
Val Asp Lys Arg Val  
215

```
gccaggctca gcgctcgtgc ctggacgcat cccggctatg cagtcccagt ccagggcagc 840  
aaggcaggcc ccgtctgcct cttcacccgg aggccctctgc ccgccccact catgctcagg 900  
gagagggtct tctggcttt tccccaggct ctgggcagggc acaggctagg tgcccctaac 960  
ccagggccctg cacacaaaagg ggcaggtgct gggctcagac ctgccaagag ccatatccgg 1020  
gaggaccctg cccctgacct aagcccaccc caaaggccaa actctccact ccctcagctc 1080  
ggacaccccttc ttcctccca gattccagta actcccaatc ttctctctgc a gag ccc 1137  
Glu Pro  
220
```

aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca ggtaagccag 1186  
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
225 230

cccaggcctc gccctccagc tcaaggcggg acaggtgccc tagagtagcc tgcatccagg 1246  
gacaggcccc agccgggtgc tgacacgtcc acctccatct cttcctca gca cct gaa 1303  
Ala Pro Glu  
235

ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac 1351  
 Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 240 245 250

```

acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac 1399
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
255          260          265

```

gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc 1447  
 Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
 270 275 280 · 285

```

agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg      1543
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
            305          310          315

```

ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca 1591  
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
 320 325 330

gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggtgggaccc gtggggtgcg 1644  
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys  
     335               340

agggccacat ggacagaggc cggctcgccc caccctctgc cctgagagtg accgctgtac 1704  
 caaacctctgt ccctaca ggg cag ccc cga gaa cca cag gtg tac acc ctg   1754  
     Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
     345               350               355

ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg acc tgc   1802  
 Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
     360               365               370

ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc   1850  
 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
     375               380               385

aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac   1898  
 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
     390               395               400

tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc   1946  
 Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser  
     405               410               415

agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct   1994  
 Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
     420               425               430               435

ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa   2042  
 Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
     440               445               450

tgagtgcgac ggccggcaag ccccgctccc gaatt                           2077

<210> 89  
 <211> 470  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Designed heavy  
       chain of humanized anti-Fas antibody

<400> 89  
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
     -15               -10               -5

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
     -1      1               5               10

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
 15 20 25

Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Ala Pro Gly Gln Arg Leu  
 30 35 40 45

Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn  
 50 55 60

Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ala Ser  
 65 70 75

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val  
 80 85 90

Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp  
 95 100 105

Val Trp Gly Glu Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
 110 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
 130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
 145 150 155

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
 160 165 170

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
 175 180 185

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
 190 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro  
 210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
 225 230 235

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 240 245 250

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
 255 260 265

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
 270 275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
 290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
                   305                  310                  315  
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
                   320                  325                  330  
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
                   335                  340                  345  
 Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
                   350                  355                  360                  365  
 Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
                   370                  375                  380  
 Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
                   385                  390                  395  
 Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
                   400                  405                  410  
 Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
                   415                  420                  425  
 Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
                   430                  435                  440                  445  
 Ser Leu Ser Pro Gly Lys  
                   450

<210> 90  
 <211> 20  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Sequencing  
 primer for a DNA encoding the heavy chain of a  
 humanized anti-Fas antibody

<400> 90  
 acagccggga aggtgtgcac

20

<210> 91  
 <211> 20  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Sequencing  
 primer for a DNA encoding the heavy chain of a  
 humanized anti-Fas antibody

<400> 91  
agacaccctc cctccctgtg

20

<210> 92  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 92  
gtgcagggcc tgggttaggg

20

<210> 93  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 93  
gcacggtgtgg catgtgtgag

20

<210> 94  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 94  
gttttgggggg gaagaggaag

20

<210> 95  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a

## humanized anti-Fas antibody

&lt;400&gt; 95

ccagtcctgg tgcaggacgg

20

&lt;210&gt; 96

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

&lt;400&gt; 96

cctgtggttc tcggggctgc

20

&lt;210&gt; 97

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

&lt;400&gt; 97

cgtggtcttg tagttgttct

20

&lt;210&gt; 98

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

&lt;400&gt; 98

cttcctcttc cccccaac

20

&lt;210&gt; 99

&lt;211&gt; 20

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 99

ccgtcctgca ccaggactgg

20

<210> 100

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 100

gcagccccga gaaccacagg

20

<210> 101

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 101

agaacaacta caagaccacg

20

<210> 102

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 102

gcctgacatc tgaggactc

19

<210> 103

<211> 19

<212> DNA

<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 103  
gagtcctcag atgtcaggc

19

<210> 104  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 104  
gagcagtaact cgttgctgcc gcgcgcgcgc ccag

34

<210> 105  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 105  
ggtatggctg attaatgatc aatg

24

<210> 106  
<211> 768  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Designed DNA encoding the light chain of a humanized anti-Fas antibody

<220>  
<221> CDS  
<222> (40)..(753)

<220>  
<221> mat peptide  
<222> (100)..(753)

<220>  
<221> sig peptide  
<222> (40)..(99)

<400> 106

cccaagctta agaaggcatcc tctcatctag ttctcagag atg gag aca gac aca 54  
Met Glu Thr Asp Thr  
-20

atc ctg cta tgg gtg ctg ctg ctc tgg gtt cca ggc tcc act ggt gag 102  
 Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Glu  
 -15 -10 -5 -1 1

att gtg ctc acc caa tct cca ggt act ttg tct ctg tct cca ggg gag	150
Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu	
5                            10                            15                    -	

```

agg gcc acc ctc tcc tgc aag gcc agc caa agt gtt gat tat gat ggt      198
Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp Gly
          20           25           30

```

gat agt tat atg aac tgg tac caa cag aaa cca gga cag gca ccc aga 246  
 Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg  
           35                40                45

ctc ctc atc tat gct gca tcc aat ctc gaa tct ggg atc cca gac agg 294  
Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Ile Pro Asp Arg  
50 55 60 65

ttt agt ggc agt ggg tct ggg aca gac ttc acc ctc acc atc tct cgt 342  
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg  
70 75 80

ctg gag ccg gag gat ttt gca gtc tat tac tgt cag caa agt aat gag 390  
 Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Asn Glu  
                   85              90              95

gat	cct	cg	acg	tcc	gg	caa	ggc	acc	aag	ctg	gaa	atc	aaa	cg	act	438
Asp	Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Thr	
100								105						110		

```

gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag ttg      486
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu
   115          120          125

```

```

aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc      534
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro
130          135          .           140          145

```

```

aga gag gcc aaa gta cag tgg aaa gtg gat aac gcc ctc caa tcg ggt      582
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly
150          155          160

```

aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc tac 630

Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr  
 165 170 175

agc ctc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac 678  
 Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His  
 180 185 190

aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc 726  
 Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val  
 195 200 205

aca aag agc ttc aac agg gga gag tgt tagtaagaat tcggg 768  
 Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 210 215

<210> 107

<211> 238

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed light  
 chain of humanized anti-Fas antibody

<400> 107

Met Glu Thr Asp Thr Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
 -20 -15 -10 -5

Gly Ser Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser  
 -1 1 5 10

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser  
 15 20 25

Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro  
 30 35 40

Gly Gln Ala Pro Arg Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
 45 50 55 60

Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
 65 70 75

Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys  
 80 85 90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Lys Leu  
 95 100 105

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro  
 110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
 125                   130                   135                   140

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn  
 145                   150                   155

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
 160                   165                   170

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
 175                   180                   185

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
 190                   195                   200

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys -  
 205                   210                   215

<210> 108

<211> 768

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (40)..(753)

<220>

<221> mat peptide

<222> (100)..(753)

<220>

<221> sig peptide

<222> (40)..(99)

<220>

<223> Description of Artificial Sequence: Designed DNA  
 encoding the light chain of a humanized anti-Fas  
 antibody

<400> 108

cccaagctta agaaggatcc tctcatctag ttctcagag atg gag aca gac aca       54  
 Met Glu Thr Asp Thr  
 -20

atc ctg cta tgg gtg ctg ctg tgg gtt cca ggc tcc act ggt gag       102  
 Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Glu  
 -15                   -10                  -5                   -1    1

att gtg ctc acc caa tct cca ggt act ttg tct ctg tct cca ggg gag   150  
 Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu  
 5                   10                   15

agg gcc acc ctc tcc tgc aag gcc agc caa agt gtt gat tat gat ggt Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp Gly	198
20 25 30	
gat agt tat atg aac tgg tac caa cag aaa cca gga cag gca ccc aga Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg	246
35 40 45	
ctc ctc atc tat gct gca tcc aat ctc gaa tct ggg atc cca gac agg Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Ile Pro Asp Arg	294
50 55 60 65	
ttt agt ggc agt ggg tct ggg aca gac ttc acc ctc acc atc cat cct Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile His Pro	342
70 75 80	
gtg gag gag gag gat gct gca acc tat tac tgt cag caa agt aat gag Val Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu	390
85 90 95	
gat cct cg <sup>g</sup> acg ttc ggt caa gg <sup>c</sup> acc aag ctg gaa atc aaa cg <sup>g</sup> act Asp Pro Arg Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr	438
100 105 110	
gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag ttg Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu	486
115 120 125	
aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro	534
130 135 140 145	
aga gag gcc aaa gta cag tgg aaa gtg gat aac gcc ctc caa tcg ggt Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly	582
150 155 160	
aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc tac Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr	630
165 170 175	
agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His	678
180 185 190	
aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val	726
195 200 205	
aca aag agc ttc aac agg gga gag tgt tagtaagaat tcggg Thr Lys Ser Phe Asn Arg Gly Glu Cys	768
210 215	

&lt;211&gt; 238

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: Designed light chain of humanized anti-Fas antibody

&lt;400&gt; 109

Met	Glu	Thr	Asp	Thr	Ile	Leu	Leu	Trp	Val	Leu	Leu	Leu	Trp	Val	Pro
-20															-5

-15

-10

Gly	Ser	Thr	Gly	Glu	Ile	Val	Leu	Thr	Gln	Ser	Pro	Gly	Thr	Leu	Ser
-1	1								5					10	

Leu	Ser	Pro	Gly	Glu	Arg	Ala	Thr	Leu	Ser	Cys	Lys	Ala	Ser	Gln	Ser
15								20						25	

Val	Asp	Tyr	Asp	Gly	Asp	Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Pro
30									35					40	

Gly	Gln	Ala	Pro	Arg	Leu	Leu	Ile	Tyr	Ala	Ala	Ser	Asn	Leu	Glu	Ser
45									50			55		60	

Gly	Ile	Pro	Asp	Arg	Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr
65									70					75	

Leu	Thr	Ile	His	Pro	Val	Glu	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys
80									85				90	

Gln	Gln	Ser	Asn	Glu	Asp	Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu
95								100					105		

Glu	Ile	Lys	Arg	Thr	Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro
110								115					120		

Ser	Asp	Glu	Gln	Leu	Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu
125									130			135		140	

Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn
145									150					155	

Ala	Leu	Gln	Ser	Gly	Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser
160								165					170		

Lys	Asp	Ser	Thr	Tyr	Ser	Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala
175									180				185		

Asp	Tyr	Glu	Lys	His	Lys	Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly
190								195					200		

Leu	Ser	Ser	Pro	Val	Thr	Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys	
205								210				215		

<210> 110

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the light chain of a humanized anti-Fas antibody

<400> 110

ggtagatgtt tgctcaccca atctccagg

29

<210> 111

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the light chain of a humanized anti-Fas antibody

<400> 111

cctggagatt gggtagcac aatctcacc

29

<210> 112

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the light chain of a humanized anti-Fas antibody

<400> 112

ccatctctcg tctggagccg gaggatttg c

31

<210> 113

<211> 31

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the light chain of a humanized anti-Fas antibody

<400> 113

gcaaaatcct ccggctccag acgagagatg g

31

<210> 114  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the light chain of a humanized anti-Fas antibody

<400> 114  
caaggcacca agctggaaat caaacggact g

31

<210> 115  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the light chain of a humanized anti-Fas antibody

<400> 115  
cagtccgtt gattccagc ttgggtgcctt g

31

<210> 116  
<211> 2071  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Designed DNA encoding the heavy chain of a humanized anti-Fas antibody

<220>  
<221> sig peptide  
<222> (21)..(77)

<220>  
<221> intron  
<222> (735)..(1125)

<220>  
<221> intron  
<222> (1171)..(1288)

<220>  
<221> intron  
<222> (1619)..(1715)

<220>  
<221> exon  
<222> (21) .. (734)

<220>  
<221> exon  
<222> (1126) .. (1170)

<220>  
<221> exon  
<222> (1289) .. (1618)

<220>  
<221> exon  
<222> (1716) .. (2036)

<220>  
<221> mat peptide  
<222> (78) .. (734)

<220>  
<221> mat peptide  
<222> (1126) .. (1170)

<220>  
<221> mat peptide  
<222> (1289) .. (1618)

<220>  
<221> mat peptide  
<222> (1716) .. (2036)

<220>  
<221> CDS  
<222> (21) .. (734)

<220>  
<221> CDS  
<222> (1126) .. (1170)

<220>  
<221> CDS  
<222> (1289) .. (1618)

<220>  
<221> CDS  
<222> (1716) .. (2036)

<400> 116  
aagcttggct tgacctcacc atg gga tgg agc tgt atc atc ctc ttc ttg gta 53  
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val  
-15 -10

gca aca gct aca ggt gtc cac tct cag gtc caa ctg gtg cag tct ggg 101

Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu Val Gln Ser Gly  
                   -5                  -1          1                     5  
  
 gct gag gtc aag aag cct ggg gct tca gtg aag gtg tcc tgc aag gct 149  
 Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala  
                   10                  15                     20  
  
 tct ggc tac acc ttc acc agc tac tgg atg cag tgg gta aaa cag gcc 197  
 Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Ala  
                   25                  30                  35                 40  
  
 cct gga cag ggc ctt gag tgg atg gga gag att gat cct tct gat agc 245  
 Pro Gly Gln Gly Leu Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser  
                   45                  50                  55  
  
 tat act aac tac aat caa aag ttc aag ggc aag gcc aca ttg act gta 293  
 Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val  
                   60                  65                  70  
  
 gac aca tcc act agc aca gcc tac atg gag ctc agc agc ctg aga tct 341  
 Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser  
                   75                  80                  85  
  
 gag gac acg gcg gtc tat tac tgt gca aga aat agg gac tat agt aac 389  
 Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn  
                   90                  95                  100  
  
 aac tgg tac ttc gat gtc tgg ggc gaa ggg acc ctg gtc acc gtc tcc 437  
 Asn Trp Tyr Phe Asp Val Trp Gly Glu Gly Thr Leu Val Thr Val Ser  
                   105                  110                  115                 120  
  
 tca gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc tcc 485  
 Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser  
                   125                  130                  135  
  
 aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag gac 533  
 Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp  
                   140                  145                  150  
  
 tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc ctg acc 581  
 Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr  
                   155                  160                  165  
  
 agc ggc gtg cac acc ttc ccg gct gtc cta cag tcc tca gga ctc tac 629  
 Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr  
                   170                  175                  180  
  
 tcc ctc agc agc gtg gtg acc gtg ccc tcc agc agc ttg ggc acc cag 677  
 Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln  
                   185                  190                  195                 200  
  
 acc tac atc tgc aac gtg aat cac aag ccc agc aac acc aag gtg gac 725  
 Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val Asp  
                   205                  210                  215

aag aga gtt ggtgagagggc cagcacaggg agggagggtg tctgctggaa 774  
 Lys Arg Val  
  
 gccaggctca gcgcctcctgc ctggacgcattccggctatgc cagtcggcacttgc cccggctatgc cagtcggcacttgc ccagggcaggc 834  
 aaggcaggcc ccgtctgcct cttcacccgg aggcctctgc ccgcggccact catgctcagg 894  
 gagagggtct tctggctttt tccccaggct ctgggcaggc acaggctagg tgcccctaac 954  
 ccaggccctg cacacaaagg ggcaggtgct gggctcagac ctgccaagag ccatatccgg 1014  
 gaggaccctg cccctgacact aagcccaccc caaaggccaa actctccact ccctcagctc 1074  
 ggacaccccttc tctcctccca gattccagta actcccaatc ttctctctgc a gag ccc 1131  
Glu Pro  
220  
  
 aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca ggtaagccag 1180  
 Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
225230  
  
 cccaggcctc gccctccagc tcaaggcggg acaggtgccccc tagagtaggcc tgcatccagg 1240  
  
 gacaggcccc agccgggtgc tgacacgtcc acctccatct cttcctca gca cct gaa 1297  
Ala Pro Glu  
235  
  
 ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac 1345  
 Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
240245250  
  
 acc ctc atg atc tcc ccg acc cct gag gtc aca tgc gtg gtg gtg gac 1393  
 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
255260265  
  
 gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc 1441  
 Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
270275280285  
  
 gtg gag gtg cat aat gcc aag aca aag ccg ccg gag gag cag tac aac 1489  
 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
290295300  
  
 agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg 1537  
 Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
305310315  
  
 ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca 1585  
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
320325330  
  
 gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggtgggaccc gtggggtgcg 1638  
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys  
335340

agggccacat ggacagaggc cggctcgcc caccctctgc cctgagagtg accgctgtac 1698  
 caacctctgt ccctaca ggg cag ccc cga gaa cca cag gtg tac acc ctg 1748  
                   Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
                   345                  350                  355  
 ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg acc tgc 1796  
 Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
                   360                  365                  370  
 ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc 1844  
 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
                   375                  380                  385  
 aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac 1892  
 Asn Gly Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
                   390                  395                  400  
 tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc 1940  
 Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser  
                   405                  410                  415  
 agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct 1988  
 Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
                   420                  425                  430                  435  
 ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa 2036  
 Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
                   440                  445                  450  
 tgagtgcgac ggccggcaag ccccgctccc gaatt 2071

<210> 117  
 <211> 470  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Designed heavy  
       chain of humanized anti-Fas antibody

<400> 117  
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
                   -15                  -10                  -5  
 Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
                   -1      1                  5                  10  
 Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
                   15                  20                  25

Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Ala Pro Gly Gln Gly Leu  
 30 35 40 45

Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn  
 50 55 60

Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Thr Ser  
 65 70 75

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val  
 80 85 90

Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp  
 95 100 105

Val Trp Gly Glu Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
 110 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
 130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
 145 150 155

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
 160 165 170

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
 175 180 185

Val Thr Val Pro Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
 190 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro  
 210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
 225 230 235

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 240 245 250

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
 255 260 265

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
 270 275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
 290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
 305 310 315

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
 320 325 330  
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
 335 340 345  
 Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
 350 355 360 365  
 Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
 370 375 380  
 Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
 385 390 395  
 Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
 400 405 410  
 Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
 415 420 425  
 Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
 430 435 440 445  
 Ser Leu Ser Pro Gly Lys  
 450

<210> 118  
 <211> 30  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR primer to  
 amplify a fragment of a DNA encoding the heavy  
 chain of a humanized anti-Fas antibody

<400> 118  
 caggcccctg gacagggcct tgagtggatg

30

<210> 119  
 <211> 30  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR primer to  
 amplify a fragment of a DNA encoding the heavy  
 chain of a humanized anti-Fas antibody

<400> 119  
 catccactca aggccctgtc caggggcctg

30

<210> 120  
<211> 39  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 120  
gctgagctcc atgtaggctg tgcttagtgga tgtgtctac

39

<210> 121  
<211> 33  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a DNA fragment including SR alpha promoter

<400> 121  
tgcacgcgtg gctgtggaat gtgtgtcagt tag

33

<210> 122  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a DNA fragment including SR alpha promoter

<400> 122  
tccgaagctt ttagagcaga agtaaacatt c

31

<210> 123  
<211> 36  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a DNA fragment including SR alpha promoter

<400> 123  
aaagcggccg ctgctagctt ggctgtggaa tgtgtg

36

<210> 124  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a DNA encoding the kappa light chain of human immunoglobulin

<400> 124  
aagcttatgg acatgagggt ccccgctctg ctcc 34

<210> 125  
<211> 729  
<212> DNA  
<213> Homo sapiens

<400> 125  
aagcttatgg acatgagggt ccccgctctg ctcctgggc tcctgctact ctggctccga 60  
gggccagat gtgacatcca gatgacccag tctccatcct ccctgtctgc atctgttagga 120  
gacagagtca ccatcacttg ccggcaagt cagagcatta gcagctattt aaattggat 180  
cagcagaaac cagggaaagc ccctaagctc ctgatctatg ctgcattccag tttgcaaagt 240  
ggggtcccatt caaggttcag tggcagtgga tctggacag atttcactct caccatcagc 300  
agtctgcaac ctgaagattt tgcaacttac tactgtcaac agagttacag taccctcga 360  
acgttcggcc aaggcacaa ggtggaaatc aaacgaactg tggctgcacc atctgtcttc 420  
atcttcccgc catctgatga gcagttgaaa tctggaaactg cctctgttgt gtgcctgctg 480  
aataacttct atcccgaga ggccaaagta cagtggagg tggataacgc cctccaatcg 540  
ggtaactccc aggagagtgt tacagagcag gacagcaagg acagcaccta cagcctcagc 600  
agcacccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc 660  
acccatcagg gcctgagctc gcccgtcaca aagagctca acaggggaga gtgttagtaa 720  
gaattcggg 729

<210> 126  
<211> 767  
<212> DNA  
<213> Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Designed DNA  
encoding the light chain of a humanized anti-Fas  
antibody

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (39)..(752)

&lt;220&gt;

&lt;221&gt; mat peptide

&lt;222&gt; (99)..(752)

&lt;220&gt;

&lt;221&gt; sig peptide

&lt;222&gt; (39)..(98)

&lt;400&gt; 126

ccaagcttaa	gaaggcatcct	ctcatctagt	tctcagag	atg	gag	aca	gac	aca	atc	56
				Met	Glu	Thr	Asp	Thr	Ile	
				-20						-15

ctg	cta	tgg	gtg	ctg	ctc	tgg	gtt	cca	ggc	tcc	act	ggt	gac	att	104
Leu	Leu	Trp	Val	Leu	Leu	Leu	Trp	Val	Pro	Gly	Ser	Thr	Gly	Asp	Ile
-10									-5			-1		1	

gtg	ctc	acc	caa	tct	cca	tcc	ctg	tct	gca	tct	gta	gga	gac	aga	152
Val	Leu	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg
5									10			15			

gtc	acc	atc	act	tgc	aag	gcc	agc	caa	agt	gtt	tat	gat	ggt	gat	200
Val	Thr	Ile	Thr	Cys	Lys	Ala	Ser	Gln	Ser	Val	Asp	Tyr	Asp	Gly	Asp
20									25			30			

agt	tat	atg	aac	tgg	tac	caa	cag	aaa	cca	gga	aag	gca	ccc	aag	ctc	248
Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	
35									40			45		50		

ctc	atc	tat	gct	gca	tcc	aat	ttg	gaa	agt	ggg	gtc	cca	tca	agg	ttc	296
Leu	Ile	Tyr	Ala	Ala	Ser	Asn	Leu	Glu	Ser	Gly	Val	Pro	Ser	Arg	Phe	
									55			60		65		

agt	gga	agt	gga	tct	ggg	aca	gat	ttt	act	ctc	acc	atc	agc	agc	ctg	344
Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu	
70									75			80				

cag	cct	gaa	gat	ttt	gca	acc	tac	tac	tgt	cag	caa	agt	aac	gag	gat	392
Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Asn	Glu	Asp	
85									90			95				

cct	cgg	acg	ttc	ggc	caa	ggc	acc	aag	gtg	gaa	atc	aaa	cgg	act	gtg	440
Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr	Val	
100									105			110				

gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag ttg aaa	488
Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys	
115 120 125 130	
tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc aga	536
Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg	
135 140 145	
gag gcc aaa gta cag tgg aaa gtg gat aac gcc ctc caa tcg ggt aac	584
Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn	
150 155 160	
tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc tac agc	632
Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser	
165 170 175	
ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac aaa	680
Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys	
180 185 190	
gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc aca	728
Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr	
195 200 205 210	
aag agc ttc aac agg gga gag tgt tagtaagaat tcggg	767
Lys Ser Phe Asn Arg Gly Glu Cys	
215	

&lt;210&gt; 127

&lt;211&gt; 238

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;223&gt; Description of Artificial Sequence: Designed light chain of humanized anti-Fas antibody

&lt;400&gt; 127

Met Glu Thr Asp Thr Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro			
-20	-15	-10	-5

Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser	
-1 1 5 10	

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser	
15 20 25	

Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro	
30 35 40	

Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser	
45 50 55 60	

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
 65 70 75

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys  
 80 85 90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Lys Val  
 95 100 105

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro  
 110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
 125 130 135 140

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn  
 145 150 155

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
 160 165 170

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
 175 180 185

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
 190 195 200

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 205 210 215

<210> 128  
 <211> 767  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Designed DNA  
 encoding the light chain of a humanized anti-Fas  
 antibody

<220>  
 <221> CDS  
 <222> (39)..(752)

<220>  
 <221> mat peptide  
 <222> (99)..(752)

<220>  
 <221> sig peptide  
 <222> (39)..(98)

<400> 128



Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys  
180 185 190

gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc aca	728
Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr	
195                   200                   205                   210	

aag agc ttc aac agg gga gag tgt tagtaagaat tcggg  
Lys Ser Phe Asn Arg Gly Glu Cys  
215

```
<210> 129  
<211> 238  
<212> PRT  
<213> Artificial Sequence
```

<220>  
<223> Description of Artificial Sequence: Designed light  
chain of humanized anti-Fas antibody

<400> 129  
Met Glu Thr Asp Thr Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
-20 -15 -10 -5

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser  
15 20 25

Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro  
30 35 40

Gly Gln Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
45 50 55 60

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
                   65                   70                   75

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys  
 -80 85 ; 90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Lys Val  
95 100 105

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro  
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
125 130 135 140

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn  
145 150 155

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
 160 165 170

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
 175 180 185

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
190 195 200

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
205 210 215

<210> 130

211 <211> 778

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed DNA encoding the light chain of a humanized anti-Fas antibody

<220>

<221> CDS

<222> (39) .. (752)

<220>

<221> mat peptide

<222> (99) .. (752)

<220>

<221> sig peptide  
  222- (30) (88)

<222> (39)..(98)

<400> 130  
aaaaaaa

ccaagcttaa gaagcatccc cccatcttgc tcccaagtag atg gag uca gac uca i...  
Met Glu Thr Asp Thr Ile  
-20 -15

ctg cta tgg gtg ctg ctg ctc tgg gtt cca ggc tcc act ggt gac att 104  
 Leu Leu Trp Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Asp Ile  
 -10 -5 -1 1

gtg ctc acc caa tct cca tcc tcc ctg tct gca tct gta gga gac aga 152  
 Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg  
               5                  10                  15

gtc acc atc act tgc aag gcc agc caa agt gtt gat tat gat ggt gat	200
Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp Gly Asp	
20 25 30	

agt tat atg aac tgg tac caa cag aaa cca gga aag gca ccc aaa ctc 248

Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu	35	40	45	50
ctc	atc	tac	gct	gca	tcc	aat	ttg	gaa	tca	ggg	atc	cca	tca	agg	ttc				296
Leu	Ile	Tyr	Ala	Ala	Ser	Asn	Leu	Glu	Ser	Gly	Ile	Pro	Ser	Arg	Phe				
							55		60					65					
agt	gga	agt	gga	tct	ggg	aca	gat	ttt	act	ctc	acc	atc	agc	agc	ctg				344
Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu				
							70		75				80						
cag	cct	gag	gat	ttt	gca	acc	tat	tac	tgt	cag	caa	agt	aat	gag	gat				392
Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Asn	Glu	Asp				
							85		90				95						
cct	cg	acg	ttc	ggt	caa	ggc	acc	aag	gtg	gaa	atc	aaa	cg	act	gtg				440
Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr	Val				
							100		105				110						
gct	gca	cca	tct	gtc	ttc	atc	ttc	ccg	cca	tct	gat	gag	cag	ttg	aaa				488
Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys				
							115		120				125		130				
tct	gga	act	gcc	tct	gtt	gtg	tgc	ctg	ctg	aat	aac	ttc	tat	ccc	aga				536
Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg				
							135		140				145						
gag	gcc	aaa	gta	cag	tgg	aag	gtg	gat	aac	gcc	ctc	caa	tcg	ggt	aac				584
Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn				
							150		155				160						
tcc	cag	gag	agt	gtc	aca	gag	cag	gac	agc	aag	gac	agc	acc	tac	agc				632
Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser				
							165		170				175						
ctc	agc	agc	acc	ctg	acg	ctg	agc	aaa	gca	gac	tac	gag	aaa	cac	aaa				680
Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys				
							180		185				190						
gtc	tac	gcc	tgc	gaa	gtc	acc	cat	cag	ggc	ctg	agc	tcg	ccc	gtc	aca				728
Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr				
							195		200				205	:	210				
aag	agc	tcc	aac	agg	gga	gag	tgt	tagtaagaat	tcgggaagcc	gaattc									778
Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys												
							215												

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<210> 131  
<211> 238  
<212> PRT  
<213> Artificial Sequence
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<220>

<223> Description of Artificial Sequence: Designed light chain of humanized anti-Fas antibody

<400> 131  
 Met Glu Thr Asp Thr Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
 -20 -15 -10 -5  
 Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser  
 -1 1 5 10  
 Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser  
 15 20 25  
 Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro  
 30 35 40  
 Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
 45 50 55 60  
 Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
 65 70 75  
 Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys  
 80 85 90  
 Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Lys Val  
 95 100 105  
 Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro  
 110 115 120  
 Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
 125 130 135 140  
 Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn  
 145 150 155  
 Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
 160 165 170  
 Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
 175 180 185  
 Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
 190 195 200  
 Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
 205 210 215

<210> 132  
 <211> 41  
 <212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 132

aggggaggatg gagattgggt gagcacaatg tcaccagtgg a

41

<210> 133

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 133

attgtgctca cccaatctcc atcctccctg tctgcatct

39

<210> 134

<211> 42

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 134

atcaacactt tggctggcct tgcaagtgtat ggtgactctg tc

42

<210> 135

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 135

ccatcacttg caaggccagc caaagtgttg attatgatgg

40

<210> 136  
<211> 48  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 136  
agtttcgaga ttggatgcag catagatgag gagtttgggt gcctttcc 48

<210> 137  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 137  
cccaagctcc tcatctatgc tgcatccaat ttggaaaatg gggtc 45

<210> 138  
<211> 44  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 138  
ttggccgaac gttcgaggat cctcgtaact ctgttgacag tagt 44

<210> 139  
<211> 44  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 139

actactgtca acagagtaac gaggatcctc gaacgttcg 44  
ccaa

<210> 140  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the light chain  
of a humanized anti-Fas antibody

<400> 140  
ctcatctatg ctgcattcaa tttggaaagt gggatccat caagg 45

<210> 141  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the light chain  
of a humanized anti-Fas antibody

<400> 141  
attggatgca gcatacatga ggagcttggg tgccctgtcct ggttt 45

<210> 142  
<211> 2073  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Designed DNA  
encoding the heavy chain of a humanized anti-Fas  
antibody

<220>  
<221> sig peptide  
<222> (23)..(79)

<220>  
<221> intron  
<222> (737)..(1127)

<220>  
<221> intron  
<222> (1173)..(1290)

<220>  
<221> intron  
<222> (1621)..(1717)

<220>  
<221> exon  
<222> (23)..(736)

<220>  
<221> exon  
<222> (1128)..(1172)

<220>  
<221> exon  
<222> (1291)..(1620)

<220>  
<221> exon  
<222> (1718)..(2038)

<220>  
<221> mat peptide  
<222> (80)..(736)

<220>  
<221> mat peptide  
<222> (1128)..(1172)

<220>  
<221> mat peptide  
<222> (1291)..(1620)

<220>  
<221> mat peptide  
<222> (1718)..(2038)

<220>  
<221> CDS  
<222> (23)..(736)

<220>  
<221> CDS  
<222> (1128)..(1172)

<220>  
<221> CDS  
<222> (1291)..(1620)

<220>  
<221> CDS  
<222> (1718)..(2038)

<400> 142

ccaagcttgg cttgaccta cc atg gga tgg agc tgt atc atc ctc ttc ttg	52
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu	
-15	-10
gta gca aca gct aca ggt gtc cat tct cag gtc caa ctg gtg cag tct	100
Val Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu Val Gln Ser	
-5                  -1          1                  5	
ggg gct gag gtc aag aag cct ggg gct tca gtg aag gtg tcc tgc aag	148
Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys	
10                  15                  20	
gct tct ggc tac acc ttc acc agc tac tgg atg cag tgg gta aaa cag	196
Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Met Gln Trp Val Lys Gln	
25                  30                  35	
gcc cct gga cag gga ctt gag tgg atg gga gag att gat cct tct gat	244
Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Glu Ile Asp Pro Ser Asp	
40                  45                  50                  55	
agc tat act aac tac aat caa aag ttc aag ggc aag gcc aca ttg act	292
Ser Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr	
60                  65                  70	
gta gac aca tcc act agc aca gcc tac atg gag ctc agc agc ctg aga	340
Val Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg	
75                  80                  85	
tct gag gac acg gcg gtc tat tac tgt gca aga aat agg gac tat agt	388
Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser	
90                  95                  100	
aac aac tgg tac ttc gat gtc tgg ggc caa ggt aca ctg gtc acc gtc	436
Asn Asn Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val	
105                110                115	
tcc tca gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc	484
Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser	
120                125                130                135	
tcc aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag	532
Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys	
140                145                150	
gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc ctg	580
Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu	
155                160                165	
acc agc ggc gtg cac acc ttc ccg gct gtc cta cag tcc tca gga ctc	628
Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu	
170                175                180	
tac tcc ctc agc agc gtg gtg acc gtg ccc tcc agc agc ttg ggc acc	676

Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr		
185					190					195							
cag	acc	tac	atc	tgc	aac	gtg	aat	cac	aag	ccc	agc	aac	acc	aag	gtg	724	
Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val		
200					205					210					215		
gac	aag	aga	gtt	ggtgagaggc	cagcacaggg	agggagggtg	tctgctggaa									776	
Asp	Lys	Arg	Val														
gccaggctca	gcgctcctgc	ctggacgcat	cccggttatg	cagtcccagt	ccagggcagc	836											
aaggcaggcc	ccgtctgcct	cttcacccgg	aggcctctgc	ccgccccact	catgctcagg	896											
gagagggtct	tctggcttt	tccccaggct	ctgggcaggg	acaggctagg	tgcccctaac	956											
ccaggccctg	cacacaaagg	ggcaggtgct	gggctcagac	ctgccaagag	ccatatccgg	1016											
gaggaccctg	cccctgacct	aagcccaccc	caaaggccaa	actctccact	ccctcagctc	1076											
ggacacccctc	tctccatccca	gattccagta	actcccaatc	ttctctctgc	a gag ccc	1133											
					Glu Pro												
					220												
aaa	tct	tgt	gac	aaa	act	cac	aca	tgc	cca	ccg	tgc	cca	ggtaagccag		1182		
Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Pro	Cys	Pro				
225					230												
cccaggcctc	gccctccagc	tcaaggcggg	acaggtgccc	tagagtagcc	tgcattcagg	1242											
gacaggcccc	agccgggtgc	tgacacgtcc	acctccatct	cttcctca	gca cct gaa	1299											
					Ala Pro Glu												
					235												
ctc	ctg	ggg	gga	ccg	tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	ccc	aag	gac	1347	
Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp		
240					245					250							
acc	ctc	atg	atc	tcc	cgg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	gac	1395	
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp		
255					260					265							
gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	ggc	1443	
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly		
270					275					280					285		
gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	ccg	cg	gag	gag	cag	tac	aac	1491	
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn		
					290					295					300		
agc	acg	tac	cgt	gtg	gtc	agc	gtc	acc	gtc	ctg	cac	cag	gac	tgg		1539	
Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp		
305					310					315							

ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca 1587  
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
     320                 325                 330

gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggtgggaccc gtggggtgcg 1640  
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys  
     335                 340

agggccacat ggacagaggc cggctcgcc caccctctgc cctgagagtg accgctgtac 1700

caaacctctgt ccctaca ggg cag ccc cga gaa cca cag gtg tac acc ctg 1750  
                   Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
                   345                 350                 355

ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg acc tgc 1798  
 Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
     360                 365                 370

ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc 1846  
 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
     375                 380                 385

aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac 1894  
 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
     390                 395                 400

tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc 1942  
 Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser  
     405                 410                 415

agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct 1990  
 Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
     420                 425                 430                 435

ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa 2038  
 Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
     440                 445                 450

tgagtgcgac ggccggcaag ccccgctccc gaatt 2073

<210> 143  
 <211> 470  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Designed heavy  
       chain of humanized anti-Fas antibody

<400> 143  
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
     -15                 -10                 -5

Val	His	Ser	Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys
-1	1					5						10			
Pro	Gly	Ala	Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe
15					20							25			
Thr	Ser	Tyr	Trp	Met	Gln	Trp	Val	Lys	Gln	Ala	Pro	Gly	Gln	Gly	Leu
30					35						40				45
Glu	Trp	Met	Gly	Glu	Ile	Asp	Pro	Ser	Asp	Ser	Tyr	Thr	Asn	Tyr	Asn
				50					55				60		
Gln	Lys	Phe	Lys	Gly	Lys	Ala	Thr	Leu	Thr	Val	Asp	Thr	Ser	Thr	Ser
					65				70				75		
Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val
						80		85				90			
Tyr	Tyr	Cys	Ala	Arg	Asn	Arg	Asp	Tyr	Ser	Asn	Asn	Trp	Tyr	Phe	Asp
					95		100				105				
Val	Trp	Gly	Gln	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys
						110	115				120				125
Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly
					130				135				140		
Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro
					145				150				155		
Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr
						160		165				170			
Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val
					175		180				185				
Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn
						190	195			200				205	
Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro
					210				215				220		
Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu
					225			230				235			
Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp
					240		245					250			
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp
					255		260				265				
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly
					270		275				280				285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
305 310 315

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
320 325 330

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
335 340 345

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
350 355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
400 405 410

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
415 420 425

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
430 435 440 445

Ser Leu Ser Pro Gly Lys  
450

<210> 144  
<211> 2073  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Designed DNA  
encoding the heavy chain of a humanized anti-Fas  
antibody

<220>  
<221> sig peptide  
<222> (23)..(79)

<220>  
<221> intron  
<222> (737)..(1127)

<220>  
<221> intron

<222> (1173)..(1290)

<220>

<221> intron

<222> (1621)..(1717)

<220>

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<222> (23)..(736)

<220>

<221> exon

<222> (1128)..(1172)

<220>

<221> exon

<222> (1291)..(1620)

<220>

<221> exon

<222> (1718)..(2038)

<220>

<221> mat peptide

<222> (80)..(736)

<220>

<221> mat peptide

<222> (1128)..(1172)

<220>

<221> mat peptide

<222> (1291)..(1620)

<220>

<221> mat peptide

<222> (1718)..(2038)

<220>

<221> CDS

<222> (23)..(736)

<220>

<221> CDS

<222> (1128)..(1172)

<220>

<221> CDS

<222> (1291)..(1620)

<220>

<221> CDS

<222> (1718)..(2038)



Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr			
185	190	195	
cag acc tac atc tgc aac gtg aat cac aag ccc agc aac acc aag gtg			724
Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val			
200	205	210	215
gac aag aga gtt ggtgagaggg cagcacaggg agggagggtg tctgctggaa			776
Asp Lys Arg Val			
gccaggctca gcgctcctgc ctggacgc cat cccggctatg cagtcccagt ccagggcagc			836
aaggcaggcc ccgtctgcct cttcacccgg aggcctctgc ccgccccact catgctcagg			896
gagagggtct tctggcttt tccccaggct ctgggcaggc acaggctagg tgccccta ac			956
ccaggccctg cacacaaagg ggcaggtgct gggctcagac ctgccaagag ccatatccgg			1016
gaggaccctg cccctgaccc aagcccaccc caaaggccaa actctccact ccctcagctc			1076
ggacacccctt ctcctccca gattccagta actcccaatc ttctctctgc a gag ccc			1133
			Glu Pro
			220
aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca ggtaagccag			1182
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro			
225	230		
cccaggcctc gccctccagc tcaaggcggg acaggtgccc tagagtagcc tgcatccagg			1242
gacaggcccc agccgggtgc tgacacgtcc acctccatct cttcctca gca cct gaa			1299
			Ala Pro Glu
			235
ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac			1347
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp			
240	245	250	
acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac			1395
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp			
255	260	265	
tg gac cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc			1443
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly			
270	275	280	285
gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac			1491
Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn			
290	295	300	
agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg			1539
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp			
305	310	315	

ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca 1587  
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
   320                   325                   330

gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggtgggaccc gtggggtgcg 1640  
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys  
   335                   340

agggccacat ggacagaggc cggctcgcc caccctctgc cctgagagt accgctgtac 1700

caaacctctgt ccctaca ggg cag ccc cga gaa cca cag gtg tac acc ctg 1750  
   Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
   345                   350                   355

ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg acc tgc 1798  
 Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
   360                   365                   370

ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc 1846  
 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
   375                   380                   385

aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac 1894  
 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
   390                   395                   400

tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc 1942  
 Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser  
   405                   410                   415

agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct 1990  
 Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
   420                   425                   430                   435

ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa 2038  
 Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
   440                   445                   450

tgagtgcgac ggccggcaag ccccgctccc gaatt 2073

<210> 145  
 <211> 470  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Designed heavy  
       chain of humanized anti-Fas antibody

<400> 145  
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
   -15                   -10                   -5

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
     -1     1               5                           10  
 Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
     15                   20                           25  
 Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Ala Pro Gly Gln Gly Leu  
     30                   35                           40                   45  
 Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn  
     50                   55                           60  
 Gln Lys Phe Lys Gly Lys Ala Thr Ile Thr Val Asp Thr Ser Thr Ser  
     65                   70                           75  
 Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val  
     80                   85                           90  
 Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp  
     95                   100                          105  
 Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
   110                   115                          120                   125  
 Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
   130                   135                          140  
 Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
   145                   150                          155  
 Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
   160                   165                          170  
 Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
   175                   180                          185  
 Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
   190                   195                          200                   205  
 Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro  
   210                   215                          220  
 Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
   225                   230                          235  
 Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
   240                   245                          250  
 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
   255                   260                          265  
 Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
   270                   275                          280                   285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
 290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
 305 310 315

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
 320 325 330

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
 335 340 345

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
 350 355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
 370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
 385 390 395

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
 400 405 410

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
 415 420 425

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
 430 435 440 445

Ser Leu Ser Pro Gly Lys  
 450

<210> 146  
 <211> 2073  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Designed DNA  
 encoding the heavy chain of a humanized anti-Fas  
 antibody

<220>  
 <221> sig peptide  
 <222> (23)..(79)

<220>  
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 <222> (737)..(1127)

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<222> (1173) .. (1290)

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<222> (23) .. (736)

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<222> (1291) .. (1620)

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<222> (1718) .. (2038)

<220>  
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<222> (80) .. (736)

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<222> (1128) .. (1172)

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<220>  
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<220>  
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<222> (1128) .. (1172)

<220>  
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<222> (1291) .. (1620)

<220>  
<221> CDS  
<222> (1718) .. (2038)



Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly Thr  
 185 190 195

cag acc tac atc tgc aac gtg aat cac aag ccc agc aac acc aag gtg 724  
 Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys Val  
 200 205 210 215

gac aag aga gtt ggtgagaggc cagcacaggg agggagggtg tctgctggaa 776  
 Asp Lys Arg Val

gccaggctca gcgctcctgc ctggacgcat cccggctatg cagtcccagt ccagggcagc 836  
 aaggcaggcc ccgtctgcct cttcacccgg aggcctctgc ccgccccact catgctcagg 896  
 gagagggtct tctggctttt tccccaggct ctgggcaggg acaggctagg tgcccctaac 956  
 ccaggccctg cacacaaagg ggcaggtgct gggctcagac ctgccaagag ccatatccgg 1016  
 gaggaccctg cccctgacct aagcccaccc caaaggccaa actctccact ccctcagctc 1076  
 ggacacccccc ttcctccca gattccagta actcccaatc ttctctctgc a gag ccc 1133  
 Glu Pro  
 220

aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca ggtaagccag 1182  
 Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
 225 230

cccaggcctc gccctccagc tcaaggcggg acaggtgcc tagagtagcc tgcatccagg 1242  
 gacaggcccc agccgggtgc tgacacgtcc acctccatct cttcctca gca cct gaa 1299  
 Ala Pro Glu  
 235

ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac 1347  
 Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
 240 245 250

acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac 1395  
 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
 255 260 265

gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc 1443  
 Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
 270 275 280 285

gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac 1491  
 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
 290 295 300

agc acg tac cgt gtg gtc agc gtc acc gtc ctg cac cag gac tgg 1539  
 Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
 305 310 315

ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca	1587
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro	
320	325
330	
gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggtgggaccc gtggggtgcg 1640	
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys	
335	340
agggccacat ggacagaggc cggctcgccc caccctctgc cctgagagtg accgctgtac 1700	
caacctctgt ccctaca ggg cag ccc cga gaa cca cag gtg tac acc ctg	1750
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu	
345	350
355	
ccc cca tcc cgg gag gag atg acc aac cag gtc agc ctg acc tgc	1798
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys	
360	365
370	
ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc	1846
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser	
375	380
385	
aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac	1894
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp	
390	395
400	
tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc	1942
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser	
405	410
415	
agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct	1990
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala	
420	425
430	
435	
ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa	2038
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys	
440	445
450	
tgagtgcgac ggccggcaag ccccgatccc gaatt	2073

<210> 147  
<211> 470  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Designed heavy chain of humanized anti-Fas antibody

<400> 147  
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
-15 -10 -5

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
     -1      1                  5                         10

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
     15                         20                         25

Thr Ser Tyr Trp Met Gln Trp Val Arg Gln Ala Pro Gly Gln Gly Leu  
     30                         35                         40                 45

Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn  
     50                         55                         60

Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Thr Ser  
     65                         70                         75

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val  
     80                         85                         90

Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp  
     95                         100                        105

Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
    110                         115                        120                 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
    130                         135                        140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
    145                         150                        155

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
    160                         165                        170

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
    175                         180                        185

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
    190                         195                        200                 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro  
    210                         215                        220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
    225                         230                        235

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
    240                         245                        250

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
    255                         260                        265

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
    270                         275                        280                 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
 290 295 300  
 Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
 305 310 315  
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
 320 325 330  
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
 335 340 345  
 Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
 350 355 360 365  
 Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
 370 375 380  
 Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
 385 390 395  
 Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
 400 405 410  
 Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
 415 420 425  
 Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
 430 435 440 445  
 Ser Leu Ser Pro Gly Lys  
 450

<210> 148  
 <211> 38  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR primer to  
 amplify a fragment of DNA encoding the heavy chain  
 of a humanized anti-Fas antibody

<400> 148  
 ccaagcttgg cttgaccta ccatggatg gagctgta 38

<210> 149  
 <211> 40  
 <212> DNA  
 <213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 149

agtgggtaaa acaggccct ggacagggac ttgagtggat

40

<210> 150

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 150

atccactcaa gtccctgtcc aggggcctgt tttacccact

40

<210> 151

<211> 64

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 151

aagaccgatg ggcccttggg ggaggctgag gagacggtga ccagtgtacc ttggccccag 60

acat

64

<210> 152

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 152

gttcaaggc aaggccacaa taactgtaga cacatccgc

39

<210> 153

<211> 39

<212> DNA  
<213> Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of a humanized anti-Fas antibody

&lt;400&gt; 153

gcggatgtgt ctacagttat tgtggccttg cccttgaac

39

&lt;210&gt; 154

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of a humanized anti-Fas antibody

&lt;400&gt; 154

agtgggtacg acaggcccct ggacaaggac ttgagtggat

40

&lt;210&gt; 155

&lt;211&gt; 40

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of a humanized anti-Fas antibody

&lt;400&gt; 155

atccactcaa gtccttgtcc aggggcctgt cgtaccact

40

&lt;210&gt; 156

&lt;211&gt; 2077

&lt;212&gt; DNA

&lt;213&gt; Artificial Sequence

&lt;220&gt;

&lt;221&gt; sig peptide

&lt;222&gt; (27)..(83)

&lt;220&gt;

&lt;221&gt; intron

&lt;222&gt; (741)..(1131)

&lt;220&gt;

<221> intron  
<222> (1177) .. (1294)

<220>  
<221> intron  
<222> (1625) .. (1725)

<220>  
<221> exon  
<222> (27) .. (740)

<220>  
<221> exon  
<222> (1132) .. (1176)

<220>  
<221> exon  
<222> (1295) .. (1624)

<220>  
<221> exon  
<222> (1722) .. (2042)

<220>  
<221> mat peptide  
<222> (84) .. (740)

<220>  
<221> mat peptide  
<222> (1132) .. (1176)

<220>  
<221> mat peptide  
<222> (1295) .. (1624)

<220>  
<221> mat peptide  
<222> (1722) .. (2042)

<220>  
<221> CDS  
<222> (27) .. (740)

<220>  
<221> CDS  
<222> (1132) .. (1176)

<220>  
<221> CDS  
<222> (1295) .. (1624)

<220>  
<221> CDS  
<222> (1722) .. (2042)

&lt;220&gt;

<223> Description of Artificial Sequence: Designed DNA  
encoding the heavy chain of humanized anti-Fas  
antibody

&lt;400&gt; 156

gggcgaaagc ttggcttgac ctcacc atg gga tgg agc tgt atc atc ctc ttc	53
Met Gly Trp Ser Cys Ile Ile Leu Phe	
-15	

ttg gta gca aca gct aca ggt gtc cac tct cag gtc caa ctg gtg cag	101
Leu Val Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu Val Gln	
-10	
-5	
-1	
1	
5	

tct ggg gct gag gtc aag aag cct ggg gct tca gtg aag gtg tcc tgc	149
Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys	
10	
15	
20	

aag gct tct ggc tac acc ttc acc agc tac tgg atg cag tgg gta cga	197
Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Met Gln Trp Val Arg	
25	
30	
35	

cag gcc cct gga cag ggc ctt gag tgg atg gga gag att gat cct tct	245
Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Glu Ile Asp Pro Ser	
40	
45	
50	

gat agc tat act aac tac aat caa aag ttc aag ggc cgg gtc aca atc	293
Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Gly Arg Val Thr Ile	
55	
60	
65	
70	

act cga gac aca tcc act agc aca gcc tac atg gag ctc agc agc ctg	341
Thr Arg Asp Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu	
75	
80	
85	

aga tct gag gac acg gcg gtc tat tac tgt gca aga aat agg gac tat	389
Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr	
90	
95	
100	

agt aac aac tgg tac ttc gat gtc tgg ggc gaa ggg acc ctg gtc acc	437
Ser Asn Asn Trp Tyr Phe Asp Val Trp Gly Glu Gly Thr Leu Val Thr	
105	
110	
115	

gtc tcc tca gcc tcc acc aag ggc cca tcg gtc ttcc ccc ctg gca ccc	485
Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro	
120	
125	
130	

tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc	533
Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val	
135	
140	
145	
150	

aag gac tac ttc ccc gaa ccg gtg acg gtg tgg aac tca ggc gcc	581
Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala	
155	
160	
165	

ctg acc agc ggc gtg cac acc ttc ccg gct gtc cta cag tcc tca gga	629
Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly	
170 175 180	
ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc agc agc ttg ggc	677
Ieu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly	
185 190 195	
acc cag acc tac atc tgc aac gtg aat cac aag ccc agc aac acc aag	725
Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys	
200 205 210	
gtg gac aag aga gtt ggtgagaggg cagcacaggg agggagggtg tctgctggaa	780
Val Asp Lys Arg Val	
215	
gccaggctca gcgcctcgtc ctggacgcatt cccggctatg cagtcccagt ccagggcagc	840
aaggcaggcc ccgtctgcct cttcacccgg aggcctctgc ccgccccact catgctcagg	900
gagagggtct tctggcttt tccccaggct ctgggcaggg acaggctagg tgcccctaac	960
ccaggccctg cacacaaaagg ggcagggtgct gggctcagac ctgccaagag ccatatccgg	1020
gaggaccctg cccctgacact aagcccaccc caaaggccaa actctccact ccctcagctc	1080
ggacacccctt tctctccca gattccagta actcccaatc ttctctctgc a gag ccc	1137
	Glu Pro
	220
aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca ggtaagccag	1186
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro	
225 230	
cccaggcctc gcctccagc tcaaggcggtt acaggtcccc tagagtagcc tgcattccagg	1246
gacaggcccc agccgggtgc tgacacgtcc acctccatct ctccctca gca cct gaa	1303
	Ala Pro Glu
	235
ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac	1351
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp	
240 245 250	
acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac	1399
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp	
255 260 265	
gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc	1447
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly	
270 275 280 285	
gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac	1495

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn			
290	295	300	
agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg		1543	
Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp			
305	310	315	
ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca		1591	
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro			
320	325	330	
gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggtgggaccc gtgggtcg 1644			
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys			
335	340		
aggccacat ggacagagggc cggctcgccc caccctctgc cctgagagtg accgctgtac 1704			
caacctctgt ccctaca ggg cag ccc cga gaa cca cag gtg tac acc ctg		1754	
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu			
345	350	355	
ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg acc tgc		1802	
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys			
360	365	370	
ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc		1850	
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser			
375	380	385	
aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac		1898	
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp			
390	395	400	
tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc		1946	
Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser			
405	410	415	
agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct		1994	
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala			
420	425	430	435
ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa		2042	
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys			
440	445	450	
tgagtgcgac ggccggcaag ccccgatccc gaatt		2077	

&lt;210&gt; 157

&lt;211&gt; 470

&lt;212&gt; PRT

&lt;213&gt; Artificial Sequence

&lt;220&gt;

<223> Description of Artificial Sequence: Designed  
heavy chain of humanized anti-Fas antibody

<400> 157

Met	Gly	Trp	Ser	Cys	Ile	Ile	Leu	Phe	Leu	Val	Ala	Thr	Ala	Thr	Gly
-15									-10						-5

Val	His	Ser	Gln	Val	Gln	Leu	Val	Gln	Ser	Gly	Ala	Glu	Val	Lys	Lys
-1	1					5							10		

Pro	Gly	Ala	Ser	Val	Lys	Val	Ser	Cys	Lys	Ala	Ser	Gly	Tyr	Thr	Phe
15						20							25		

Thr	Ser	Tyr	Trp	Met	Gln	Trp	Val	Arg	Gln	Ala	Pro	Gly	Gln	Gly	Leu
30					35				40						45

Glu	Trp	Met	Gly	Glu	Ile	Asp	Pro	Ser	Asp	Ser	Tyr	Thr	Asn	Tyr	Asn
				50					55				60		

Gln	Lys	Phe	Lys	Gly	Arg	Val	Thr	Ile	Thr	Arg	Asp	Thr	Ser	Thr	Ser
				65				70				75			

Thr	Ala	Tyr	Met	Glu	Leu	Ser	Ser	Leu	Arg	Ser	Glu	Asp	Thr	Ala	Val
				80				85				90			

Tyr	Tyr	Cys	Ala	Arg	Asn	Arg	Asp	Tyr	Ser	Asn	Asn	Trp	Tyr	Phe	Asp
				95			100					105			

Val	Trp	Gly	Glu	Gly	Thr	Leu	Val	Thr	Val	Ser	Ser	Ala	Ser	Thr	Lys
110				115					120						125

Gly	Pro	Ser	Val	Phe	Pro	Leu	Ala	Pro	Ser	Ser	Lys	Ser	Thr	Ser	Gly
					130				135				140		

Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	Lys	Asp	Tyr	Phe	Pro	Glu	Pro
				145				150				155			

Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	Leu	Thr	Ser	Gly	Val	His	Thr
				160				165				170			

Phe	Pro	Ala	Val	Leu	Gln	Ser	Ser	Gly	Leu	Tyr	Ser	Leu	Ser	Ser	Val
				175				180				185			

Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	Gln	Thr	Tyr	Ile	Cys	Asn
					190			195			200			205	

Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	Asp	Lys	Arg	Val	Glu	Pro
				210				215				220			

Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro	Ala	Pro	Glu
				225				230				235			

Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp
				240			245					250			

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
 255 260 265  
 Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
 270 275 280 285  
 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
 290 295 300  
 Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
 305 310 315  
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
 320 325 330  
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
 335 340 345  
 Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
 350 355 360 365  
 Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
 370 375 380  
 Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
 385 390 395  
 Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
 400 405 410  
 Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
 415 420 425  
 Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
 430 435 440 445  
 Ser Leu Ser Pro Gly Lys  
 450

<210> 158  
 <211> 29  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR primer to  
 amplify a fragment of DNA encoding the heavy chain  
 of humanized anti-Fas antibody

<400> 158  
 gatgcagtgg gtacgacagg cccctggac

<210> 159

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of humanized anti-Fas antibody

<400> 159

gtccaggggc ctgtcgtaacc cactgcac

29

<210> 160

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of humanized anti-Fas antibody

<400> 160

caagggccgg gtcacaatca ctcgagacac atc

33

<210> 161

<211> 33

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of humanized anti-Fas antibody

<400> 161

gatgtgtctc gagtgattgt gaccggccc ttg

33

<210> 162

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of humanized anti-Fas antibody

<400> 162

ctacaatcaa aagttcaagg

<210> 163  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing  
primer for a DNA encoding the heavy chain of  
humanized anti-Fas antibody

<400> 163  
gactatagta acaactggta c

21

<210> 164  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing  
primer for a DNA encoding the heavy chain of  
humanized anti-Fas antibody

<400> 164  
gtaccagttg ttactatagt c

21

<210> 165  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing  
primer for a DNA encoding the heavy chain of  
humanized anti-Fas antibody

<400> 165  
gcagccccagg gcccgctgtgc

20

What is claimed is:

1. A molecule having an antigen binding region specific for an epitope of the Fas antigen, said epitope being conserved between a primate and a non-primate animal.
2. The molecule of claim 1, wherein the primate is human.
3. The molecule of claim 1, wherein the non-primate is a rodent.
4. The molecule of claim 3, wherein the rodent is a mouse.
5. The molecule of claim 1, wherein the primate is human and the non-primate animal is a mouse.
6. A molecule having an antigen binding region specific for a conserved, mammalian Fas epitope.
7. An antibody produced by the hybridoma HFE7A having the accession number FERM BP-5828.
8. A molecule having at least six antibody CDR's, said antibody being specific for human Fas, wherein said CDR's have identity with the CDR's of the antibody produced by the hybridoma HFE7A having the accession number FERM BP-5828.

9. A molecule having an antigen binding region, said binding region having specificity for the antigenic determinant recognized by the antibody produced by the hybridoma HFE7A having the accession number FERM BP-5828.

10. The molecule of claim 1, which is an antibody.

11. The molecule of claim 6, which is an antibody.

12. The molecule of claim 8, which is an antibody.

13. The molecule of claim 9, which is an antibody.

14. A molecule comprising a light polypeptide chain and a heavy polypeptide chain, the heavy chain having the following formula (I):



wherein  $\text{FRH}_1$  represents an amino acid sequence having 18 to 30 amino acids,  $\text{CDRH}_1$  represents the sequence of SEQ ID NO: 2,  $\text{FRH}_2$  represents an amino acid sequence having 14 amino acids,  $\text{CDRH}_2$  represents the sequence of SEQ ID NO: 3,  $\text{FRH}_3$  represents an amino acid sequence having 32 amino acids,  $\text{CDRH}_3$  represents the sequence of SEQ ID NO: 4,  $\text{FRH}_4$  represents an amino acid sequence having 11 amino acids, and each amino acid binds to another amino acid via a peptide bond,

the light chain having the following formula (II):

-FRL<sub>1</sub>-CDRL<sub>1</sub>-FRL<sub>2</sub>-CDRL<sub>2</sub>-FRL<sub>3</sub>-CDRL<sub>3</sub>-FRL<sub>4</sub>- (II)

wherein FRL<sub>1</sub> represents an amino acid sequence having 23 amino acids, CDRL<sub>1</sub> represents the sequence of SEQ ID NO: 5, FRL<sub>2</sub> represents an amino acid sequence having 15 amino acids, CDRL<sub>2</sub> represents the sequence of SEQ ID NO: 6, FRL<sub>3</sub> represents an amino acid sequence having 32 amino acids, CDRL<sub>3</sub> represents the sequence of SEQ ID NO: 7, FRL<sub>4</sub> represents an amino acid sequence having 10 amino acids, and each amino acid binds to another amino acid via a peptide bond.

15. The molecule of claim 14, which is an antibody.

16. The antibody of any of claims 10, 11, 12, 13 or 15, which is an immunoglobulin G antibody.

17. The molecule of any of claims 1 to 6, 8, 9 or 14, which is humanized.

18. The antibody of any of claims 7, 10, 11, 12, 13 or 15, which is humanized.

19. The molecule of claim 1, which induces apoptosis in abnormal cells expressing Fas, and which inhibits apoptosis in normal cells.

20. The molecule of claim 8, which induces apoptosis in abnormal cells expressing Fas, and which inhibits apoptosis in normal cells.
21. A method to evaluate therapies for conditions in humans affected by the Fas/Fas ligand interaction, comprising introducing the molecule of claim 8 into an animal which is a model for said conditions, and evaluating said conditions exhibited by the animal.
22. A humanized molecule having an antigen binding region specific for an epitope of the Fas antigen conserved between a primate and a non-primate animal, said molecule obtained by grafting the respective CDR's from an antibody specific for said epitope of the Fas antigen into each of at least one human light chain, or fragment thereof, and at least one human heavy chain, or fragment thereof.
23. The molecule of claim 22, the antibody from which the CDR's are obtained having variable regions comprising said CDR's, and wherein said human light and heavy chains are selected on the basis of closest similarity between variable regions comprised therein and the variable regions of said antibody.
24. The molecule of claim 22, wherein significant amino acids, from framework regions comprised in an epitope recognition site

of the antibody from which the CDR's are obtained, are also grafted into said heavy and light chains to maintain structure in the epitope recognition site.

25. The molecule of any of claims 1, 6, 8 or 9 that binds a peptide comprising the amino acid sequence of SEQ ID NO: 1.

26. The molecule of any of claims 1, 6, 8 or 9 that comprises a light chain polypeptide protein selected individually from the group consisting of the amino acid sequence 1 to 218 of SEQ ID NO: 50, the amino acid sequence 1 to 218 of SEQ ID NO: 52, the amino acid sequence 1 to 218 of SEQ ID NO: 54, the amino acid sequence 1 to 218 of SEQ ID NO: 107 and the amino acid sequence 1 to 218 of SEQ ID NO: 109.

27. The molecule of any of claims 1, 6, 8 or 9 that comprises a heavy chain polypeptide protein selected individually from the group consisting of the amino acid sequence 1 to 451 of SEQ ID NO: 89 and the amino acid sequence 1 to 451 of SEQ ID NO: 117.

28. The molecule of any of claims 1, 6, 8 or 9 that comprises a light chain polypeptide protein having the amino acid sequence 1 to 218 of SEQ ID NO: 50, and a heavy chain polypeptide protein having the amino acid sequence 1 to 451 of SEQ ID NO: 89.

29. The molecule of any of claims 1, 6, 8 or 9 that comprises a light chain polypeptide protein having the amino acid sequence 1 to 218 of SEQ ID NO: 107, and a heavy chain polypeptide protein having the amino acid sequence 1 to 451 of SEQ ID NO: 117.

30. A DNA encoding a single polypeptide portion of a molecule of any of claims 1, 6, 8 or 9.

31. A DNA comprising a nucleotide sequence selected from the group consisting of the nucleotide sequence 100 to 753 of SEQ ID NO: 49, the nucleotide sequence 100 to 753 of SEQ ID NO: 51, the nucleotide sequence 100 to 753 of SEQ ID NO: 53, the nucleotide sequence 100 to 753 of SEQ ID NO: 106 and the nucleotide sequence 100 to 753 of SEQ ID NO: 108.

32. A DNA comprising a nucleotide sequence selected from the group consisting of nucleotide sequence 84 to 2042 of SEQ ID NO: 88 and nucleotide sequence 84 to 2024 of SEQ ID NO: 116.

33. A recombinant DNA vector comprising DNA encoding a light chain polypeptide protein selected individually from the group consisting of the amino acid sequence 1 to 218 of SEQ ID NO: 50, the amino acid sequence 1 to 218 of SEQ ID NO: 52, the amino acid sequence 1 to 218 of SEQ ID NO: 54, the amino acid sequence 1 to 218 of SEQ ID NO: 107 and the amino acid sequence 1 to 218 of SEQ ID NO: 109.

34. A recombinant DNA vector comprising DNA encoding a heavy chain polypeptide protein selected individually from the group consisting of the amino acid sequence 1 to 451 of SEQ ID NO: 89 and the amino acid sequence 1 to 451 of SEQ ID NO: 117.

35. A host cell transformed with a recombinant DNA vector comprising DNA encoding a light chain polypeptide protein selected individually from the group consisting of the amino acid sequence 1 to 218 of SEQ ID NO: 50, the amino acid sequence 1 to 218 of SEQ ID NO: 52, the amino acid sequence 1 to 218 of SEQ ID NO: 54, the amino acid sequence 1 to 218 of SEQ ID NO: 107 and the amino acid sequence 1 to 218 of SEQ ID NO: 109.

36. A host cell transformed with a recombinant DNA vector comprising DNA encoding a heavy chain polypeptide protein selected individually from the group consisting of the amino acid sequence 1 to 451 of SEQ ID NO: 89 and the amino acid sequence 1 to 451 of SEQ ID NO: 117.

37. A host cell transformed with at least one recombinant DNA vector comprising DNA encoding a light chain polypeptide protein and DNA encoding a heavy chain polypeptide protein, said light chain polypeptide protein comprising a sequence selected individually from the group consisting of the amino acid sequence 1 to 218 of SEQ ID NO: 50, the amino acid sequence 1 to 218 of SEQ ID NO: 52, the amino acid sequence 1 to 218 of SEQ ID NO: 54,

the amino acid sequence 1 to 218 of SEQ ID NO: 107 and the amino acid sequence 1 to 218 of SEQ ID NO: 109,

    said heavy chain polypeptide protein comprising a sequence selected individually from the group consisting of the amino acid sequence 1 to 451 of SEQ ID NO: 89 and the amino acid sequence 1 to 451 of SEQ ID NO: 117.

38. The host cell of claims 35, 36 or 37, which is mammalian.

39. An *E. coli* which is selected from the group consisting of *E. coli* pHSGMM6 SANK73697 (FERM BP-6071), *E. coli* pHSGHM17 SANK73597 (FERM BP-6072), *E. coli* pHSGHH7 SANK73497 (FERM BP-6073), *E. coli* pHSHM2 SANK 70198, *E. coli* pHSHH5 SANK 70398 (FERM BP-6272), *E. coli* pgHSL7A62 (FERM BP-6274) SANK 73397 (FERM BP-6074) and *E. coli* pgHPDHV3 SANK 70298 (FERM BP-6273).

40. A method for producing a humanized anti-Fas antibody comprising culturing the host cell of claim 37, and then recovering the humanized anti-Fas antibody from the culture.

41. An agent for the treatment or prophylaxis of conditions attributable to abnormalities of the Fas/Fas ligand system comprising, as an active ingredient, the molecule of claims 1, 6, 8 or 9 in combination with a pharmaceutically acceptable carrier.

42. An agent for the treatment or prophylaxis of conditions attributable to abnormalities of the Fas/Fas ligand system comprising, as an active ingredient, the molecule of claims 1, 6, 8 or 9 in combination with a pharmaceutically acceptable carrier, wherein said condition is selected from the group consisting of an autoimmune disease, allergy, atopy, arteriosclerosis, myocarditis, cardiomyopathy, glomerular nephritis, hypoplastic anemia, hepatitis, acquired immunodeficiency syndrome and rejection after organ transplantation.

43. An agent for the treatment or prophylaxis of conditions attributable to abnormalities of the Fas/Fas ligand system comprising, as an active ingredient, the molecule of claims 1, 6, 8 or 9 in combination with a pharmaceutically acceptable carrier, wherein said condition is an autoimmune disease selected from the group consisting of systemic lupus erythematosus, Hashimoto's disease, rheumatoid arthritis, graft versus host disease, Sjögren syndrome, pernicious anemia, Addison's disease, scleroderma, Goodpasture syndrome, Crohn's disease, autoimmune hemolytic anemia, sterility, myasthenia gravis, multiple sclerosis, Basedow's disease, thrombopenia purpura and insulin dependent diabetes mellitus.

44. An agent for the treatment or prophylaxis of conditions attributable to abnormalities of the Fas/Fas ligand system

comprising, as an active ingredient, the molecule of claims 1, 6, 8 or 9 in combination with a pharmaceutically acceptable carrier, wherein said condition is an allergy.

45. An agent for the treatment or prophylaxis of conditions attributable to abnormalities of the Fas/Fas ligand system comprising, as an active ingredient, the molecule of claims 1, 6, 8 or 9 in combination with a pharmaceutically acceptable carrier, wherein said condition is rheumatoid arthritis.

46. An agent for the treatment or prophylaxis of conditions attributable to abnormalities of the Fas/Fas ligand system comprising, as an active ingredient, the molecule of claims 1, 6, 8 or 9 in combination with a pharmaceutically acceptable carrier, wherein said condition is arteriosclerosis.

47. An agent for the treatment or prophylaxis of conditions attributable to abnormalities of the Fas/Fas ligand system comprising, as an active ingredient, the molecule of claims 1, 6, 8 or 9 in combination with a pharmaceutically acceptable carrier, wherein said condition is selected from the group consisting of myocarditis and cardiomyopathy.

48. An agent for the treatment or prophylaxis of conditions attributable to abnormalities of the Fas/Fas ligand system comprising, as an active ingredient, the molecule of claims 1, 6,

8 or 9 in combination with a pharmaceutically acceptable carrier, wherein said condition is glomerular nephritis.

49. An agent for the treatment or prophylaxis of conditions attributable to abnormalities of the Fas/Fas ligand system comprising, as an active ingredient, the molecule of claims 1, 6, 8 or 9 in combination with a pharmaceutically acceptable carrier, wherein said condition is hypoplastic anemia.

50. An agent for the treatment of prophylaxis of conditions attributable to abnormalities of the Fas/Fas ligand system comprising, as an active ingredient, the molecule of claims 1, 6, 8 or 9 in combination with a pharmaceutically acceptable carrier, wherein said condition is hepatitis.

51. An agent for the treatment or prophylaxis of conditions attributable to abnormalities of the Fas/Fas ligand system comprising, as an active ingredient, the molecule of claims 1, 6, 8 or 9 in combination with a pharmaceutically acceptable carrier, wherein said condition is selected from the group consisting of fulminant hepatitis, chronic hepatitis, viral hepatitis, hepatitis C, hepatitis B, hepatitis D and alcoholic hepatitis.

52. An agent for the treatment or prophylaxis of conditions attributable to abnormalities of the Fas/Fas ligand system comprising, as an active ingredient, the molecule of claims 1, 6,

8 or 9 in combination with a pharmaceutically acceptable carrier, wherein said condition is rejection after organ transplantation.

53. An agent for the treatment or prophylaxis of conditions attributable to abnormalities of the Fas/Fas ligand system comprising, as an active ingredient, the molecule of claims 1, 6, 8 or 9 in combination with a pharmaceutically acceptable carrier, wherein said condition is acquired immune deficiency syndrome.

54. The molecule of claim 1, 6, 8 or 9 which has a property selected from the group consisting of:  
inducing apoptosis in T cells expressing Fas;  
ameliorating autoimmune symptoms in MRL gld/gld mice;  
does not induce hepatic disorders;  
a therapeutic or prophylactic effect on fulminant hepatitis;  
a preventative effect on the onset of collagen-induced arthritis;  
inducing apoptosis in synovial cells from a rheumatoid arthritis patient.

55. The molecule of claim 1, 6, 8 or 9 which has the following properties:  
inducing apoptosis in T cells expressing Fas;  
ameliorating autoimmune symptoms in MRL gld/gld mice;  
does not induce hepatic disorders;  
a therapeutic or prophylactic effect on fulminant hepatitis;  
a preventative effect on the onset of collagen-induced arthritis;  
inducing apoptosis in synovial cells from a rheumatoid arthritis patient.

56. A method for the treatment or prophylaxis of a condition attributable to an abnormality of the Fas/Fas ligand system comprising administration of an effective, non-toxic dose of the molecule of claim 1, 6, 8 or 9.

57. A method for the treatment or prophylaxis of a condition attributable to an abnormality of the Fas/Fas ligand system comprising administration of an effective, non-toxic dose of the molecule of claim 1, 6, 8 or 9, wherein said condition is selected from the group consisting of autoimmune diseases, allergy, atopy, arteriosclerosis, myocarditis, cardiomyopathy, glomerular nephritis, hypoplastic anemia, hepatitis, acquired immunodeficiency syndrome and rejection after organ transplantation.

58. The hybridoma HFE7A having the accession number FERM BP-5828.

59. A method for producing an antibody having an antigen binding region specific for an epitope of the Fas antigen, said method comprising culturing the hybridoma HFE7A having the accession number FERM BP-5828, and harvesting expressed antibody.

60. A method for producing an antibody, said method comprising culturing the hybridoma HFE7A having the accession number FERM BP-5828, and harvesting expressed antibody.

61. A humanized anti-Fas antibody molecule capable of, in cells having an abnormality in the Fas/Fas ligand system,
- (a) inducing apoptosis by binding Fas on the cell surface, and in cells having no such abnormality,
  - (b) preventing apoptosis which would otherwise be induced by the binding of Fas ligand to the Fas antigen.
62. An antibody molecule comprising one or more heavy chain subunits having an amino acid sequence selected from the group consisting of:
- the amino acid sequence 1 to 451 of SEQ ID NO: 143;
- the amino acid sequence 1 to 451 of SEQ ID NO: 145;
- the amino acid sequence 1 to 451 of SEQ ID NO: 147; and
- the amino acid sequence 1 to 451 of SEQ ID NO: 157.
63. The antibody of claim 62, which has one or more light chain subunits having an amino acid sequence selected from the group consisting of:
- the amino acid sequence 1 to 218 of SEQ ID NO: 107;
- the amino acid sequence 1 to 218 of SEQ ID NO: 127;
- the amino acid sequence 1 to 218 of SEQ ID NO: 129; and
- the amino acid sequence 1 to 218 of SEQ ID NO: 131.
64. The antibody of claim 62, wherein the heavy chain consists essentially of the amino acid sequence 1 to 451 of SEQ ID NO: 157.

65. The antibody of claim 64, wherein the light chain consists essentially of the amino acid sequence 1 to 218 of SEQ ID NO: 107.

66. The antibody of claim 62, which consists essentially of two heavy chains and two light chains.

67. The antibody of claim 62, which consists of two heavy chains and two light chains, said heavy chains each consisting essentially of the amino acid sequence 1 to 451 of SEQ ID NO: 157, and said light chains each consisting essentially of the amino acid sequence 1 to 218 of SEQ ID NO: 107.

68. An antibody molecule, wherein one or more light chain subunits have an amino acid sequence selected from the group consisting of:

the amino acid sequence 1 to 218 of SEQ ID NO: 127;  
the amino acid sequence 1 to 218 of SEQ ID NO: 129; and  
the amino acid sequence 1 to 218 of SEQ ID NO: 131, and one or more heavy chain subunits having an amino acid sequence selected from the group consisting of:

the amino acid sequence 1 to 451 of SEQ ID NO: 143;  
the amino acid sequence 1 to 451 of SEQ ID NO: 145; and  
the amino acid sequence 1 to 451 of SEQ ID NO: 147.

69. The antibody of claim 68, wherein said light chain subunits have the amino acid sequence 1 to 218 of SEQ ID NO: 127, and one or more heavy chain subunits have the amino acid sequence 1 to 451 of SEQ ID NO: 143.

70. The antibody of claim 68, wherein said light chain subunits have the amino acid sequence 1 to 218 of SEQ ID NO: 127, and one or more heavy chain subunits have the amino acid sequence 1 to 451 of SEQ ID NO: 145.

71. The antibody of claim 68, wherein said light chain subunits have the amino acid sequence 1 to 218 of SEQ ID NO: 127, and one or more heavy chain subunits have the amino acid sequence 1 to 451 of SEQ ID NO: 147.

72. The antibody of claim 68, wherein said light chain subunits have the amino acid sequence 1 to 218 of SEQ ID NO: 129, and one or more heavy chain subunits have the amino acid sequence 1 to 451 of SEQ ID NO: 143.

73. The antibody of claim 68, wherein said light chain subunits have the amino acid sequence 1 to 218 of SEQ ID NO: 129, and one or more heavy chain subunits have the amino acid sequence 1 to 451 of SEQ ID NO: 145.

74. The antibody of claim 68, wherein said light chain subunits have the amino acid sequence 1 to 218 of SEQ ID NO: 129, and one or more heavy chain subunits have the amino acid sequence 1 to 451 of SEQ ID NO: 147.

75. The antibody of claim 68, wherein said light chain subunits have the amino acid sequence 1 to 218 of SEQ ID NO: 131, and one or more heavy chain subunits have the amino acid sequence 1 to 451 of SEQ ID NO: 143.

76. The antibody of claim 68, wherein said light chain subunits have the amino acid sequence 1 to 218 of SEQ ID NO: 131, and one or more heavy chain subunits have the amino acid sequence 1 to 451 of SEQ ID NO: 145.

77. The antibody of claim 68, wherein said light chain subunits have the amino acid sequence 1 to 218 of SEQ ID NO: 131, and one or more heavy chain subunits have the amino acid sequence 1 to 451 of SEQ ID NO: 147.

78. The antibody of claim 68, which consists essentially of two heavy chains and two light chains.

79. An agent for the prophylaxis or treatment of conditions involving an abnormality in the Fas/Fas ligand system, comprising the antibody of claim 62 as an active ingredient in combination with a pharmaceutically acceptable carrier.

80. An agent for the prophylaxis or treatment of conditions involving an abnormality in the Fas/Fas ligand system, comprising the antibody of claim 67 as an active ingredient in combination with a pharmaceutically acceptable carrier.
81. An agent for the prophylaxis or treatment of conditions involving an abnormality in the Fas/Fas ligand system, comprising the antibody of claim 68 as an active ingredient in combination with a pharmaceutically acceptable carrier.
82. A method of treatment of a condition involving an abnormality in the Fas/Fas ligand system, comprising administering a pharmaceutically effective amount of the antibody of claim 62 to a mammal in need thereof.
83. The method of claim 82, wherein the mammal is a human.
84. A method of treatment of a condition involving an abnormality in the Fas/Fas ligand system, comprising administering a pharmaceutically effective amount of the antibody of claim 67 to a mammal in need thereof.
85. The method of claim 84, wherein the mammal is a human.
86. A method of treatment of a condition involving an abnormality in the Fas/Fas ligand system, comprising administering

a pharmaceutically effective amount of the antibody of claim 68 to a mammal in need thereof.

87. The method of claim 86, wherein the mammal is a human.

88. The antibody of claim 62 that binds a peptide comprising the amino acid sequence of SEQ ID NO: 1.

89. The antibody of claim 67 that binds a peptide comprising the amino acid sequence of SEQ ID NO: 1.

90. The antibody of claim 68 that binds a peptide comprising the amino acid sequence of SEQ ID NO: 1.

91. A DNA encoding the antibody of claim 62.

92. A DNA encoding the antibody of claim 67.

93. A DNA encoding the antibody of claim 68.

94. A recombinant DNA vector comprising DNA encoding the antibody of claim 62.

95. A recombinant DNA vector comprising DNA encoding the antibody of claim 67.

96. A recombinant DNA vector comprising DNA encoding the antibody of claim 68.

97. A host cell transformed with a recombinant DNA vector comprising DNA encoding the antibody of claim 62.

98. A host cell transformed with a recombinant DNA vector comprising DNA encoding the antibody of claim 67.

99. A host cell transformed with a recombinant DNA vector comprising DNA encoding the antibody of claim 68.

100. A method for producing an anti-Fas antibody comprising culturing the host cell of claim 97, and recovering antibody from the resulting culture.

101. A method for producing an anti-Fas antibody comprising culturing the host cell of claim 98, and recovering antibody from the resulting culture.

102. A method for producing an anti-Fas antibody comprising culturing the host cell of claim 99, and recovering antibody from the resulting culture.

103. The agent of claim 79, wherein the disease is selected from the group consisting of: autoimmune diseases selected from the group consisting of systemic lupus erythematosus, Hashimoto's disease, rheumatoid arthritis, graft versus host disease, Sjögren syndrome, pernicious anemia, Addison's disease, scleroderma, Goodpasture syndrome, Crohn's disease, autoimmune hemolytic anemia, sterility, myasthenia gravis, multiple sclerosis, Basedow's disease, thrombopenia purpura and insulin dependent diabetes mellitus; allergy; rheumatoid arthritis; arteriosclerosis; myocarditis; cardiomyopathy; glomerular nephritis; hypoplastic anemia; hepatitis selected from the group consisting of fulminant hepatitis, chronic hepatitis, viral hepatitis further selected from the group consisting of hepatitis C, hepatitis B, hepatitis D and alcoholic hepatitis; and rejection after organ transplantation.

104. The agent of claim 80, wherein the disease is selected from the group consisting of: autoimmune diseases selected from the group consisting of systemic lupus erythematosus, Hashimoto's disease, rheumatoid arthritis, graft versus host disease, Sjögren syndrome, pernicious anemia, Addison's disease, scleroderma, Goodpasture syndrome, Crohn's disease, autoimmune hemolytic anemia, sterility, myasthenia gravis, multiple sclerosis, Basedow's disease, thrombopenia purpura and insulin dependent diabetes mellitus; allergy; rheumatoid arthritis; arteriosclerosis; myocarditis; cardiomyopathy; glomerular nephritis; hypoplastic anemia; hepatitis selected from the group consisting of fulminant hepatitis, chronic hepatitis, viral hepatitis further selected from the group consisting of hepatitis C, hepatitis B, hepatitis D and alcoholic hepatitis; and rejection after organ transplantation.

105. The agent of claim 81, wherein the disease is selected from the group consisting of: autoimmune diseases selected from the group consisting of systemic lupus erythematosus, Hashimoto's disease, rheumatoid arthritis, graft versus host disease, Sjögren syndrome, pernicious anemia, Addison's disease, scleroderma, Goodpasture syndrome, Crohn's disease, autoimmune hemolytic anemia, sterility, myasthenia gravis, multiple sclerosis, Basedow's disease, thrombopenia purpura and insulin dependent diabetes mellitus; allergy; rheumatoid arthritis; arteriosclerosis; myocarditis; cardiomyopathy; glomerular nephritis; hypoplastic anemia; hepatitis selected from the group consisting of fulminant hepatitis, chronic hepatitis, viral hepatitis further selected from the group consisting of hepatitis C, hepatitis B, hepatitis D and alcoholic hepatitis; and rejection after organ transplantation.

106. A transformant strain of *E. coli* selected from the group consisting of FERM BP-6512, FERM BP-6511, FERM BP-6513, FERM BP-6515, FERM BP-6514, FERM BP-6516 and FERM BP-6510.

107. A first polypeptide protein having an amino acid sequence selected from the group consisting of the amino acid sequence 1 to 218 of SEQ ID NO: 127, the amino acid sequence 1 to 218 of SEQ ID NO: 129 and the amino acid sequence 1 to 218 of SEQ ID NO: 131, together with a second polypeptide protein having an amino acid sequence selected from the group consisting of the amino acid sequence 1 to 451 of SEQ ID NO: 143, the amino acid sequence 1 to 451 of SEQ ID NO: 145 and the amino acid sequence 1 to 451 of SEQ ID NO: 147, said first polypeptide protein and said second polypeptide protein constituting a Fas-specific antibody.

108. A DNA encoding the first polypeptide according to claim  
107.

109. A DNA encoding the first polypeptide according to claim  
107, and which comprises a nucleotide sequence 99 to 752 of SEQ  
ID NO: 126.

110. A DNA encoding the first polypeptide according to claim  
107, and which comprises a nucleotide sequence 99 to 752 of SEQ  
ID NO: 128.

111. A DNA encoding the first polypeptide according to claim  
107, and which comprises a nucleotide sequence 99 to 752 of SEQ  
ID NO: 130.

112. A DNA encoding the first polypeptide according to claim  
107, and which comprises a nucleotide sequence 80 to 2038 of SEQ  
ID NO: 142.

113. A DNA encoding first polypeptide according to claim 107,  
and which comprises a nucleotide sequence 80 to 1038 of SEQ ID  
NO: 144.

114. A DNA encoding the first polypeptide according to claim  
107, and which comprises a nucleotide sequence 80 to 2038 of SEQ  
ID NO: 146.

115. A recombinant DNA vector comprising the DNA according to any one of claims 108, 109, 110, 111, 112, 113 or 114.

116. A host cell transformed with a recombinant DNA vector according to claim 115.

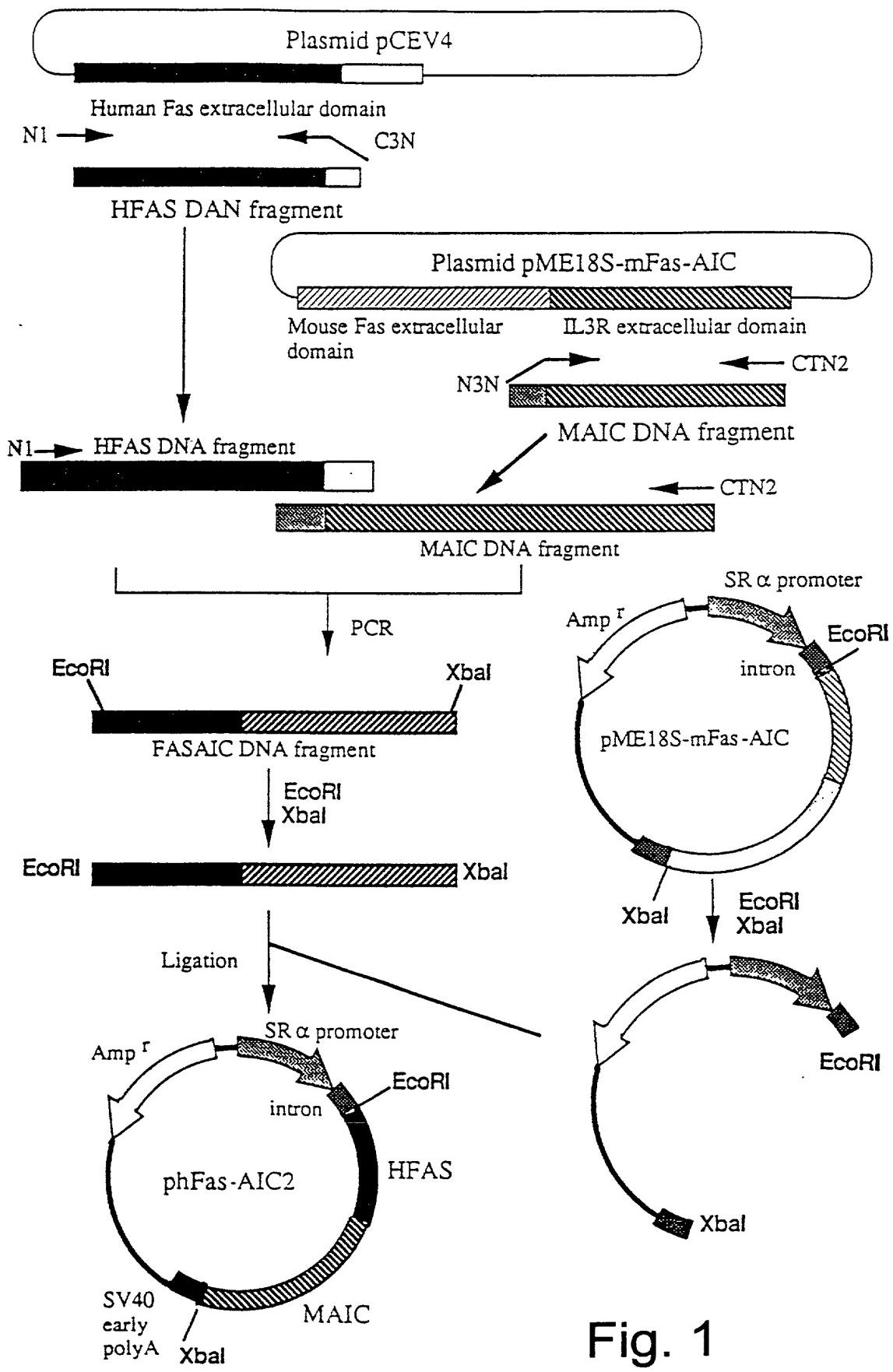
117. A host cell transformed with a recombinant DNA vector comprising the DNA of any one of claims 109, 110, 111, 112, 113 or 114, and combinations thereof.

118. A host cell transformed with a first recombinant DNA vector and a second recombinant DNA vector, said first recombinant DNA vector comprising a DNA encoding the polypeptide according to claim 107 and comprising a nucleotide sequence 99 to 752 of a SEQ ID NO selected from the group consisting of said SEQ ID NO: 126; SEQ ID NO: 128 and SEQ ID NO: 129; and said second recombinant DNA vector comprising a DNA encoding the polypeptide according to claim 107 and comprising nucleotide sequence 80 to 2038 of a SEQ ID NO selected from the group consisting of SEQ ID NO: 142, SEQ ID NO: 144 and SEQ ID NO: 146.

119. The host cell of claim 116, which is a mammalian.

ABSTRACT OF THE DISCLOSURE

Anti-Fas antibodies which are cross-reactive with mouse and human Fas and are useful in the treatment of conditions attributable to abnormalities in the Fas/Fas ligand system.



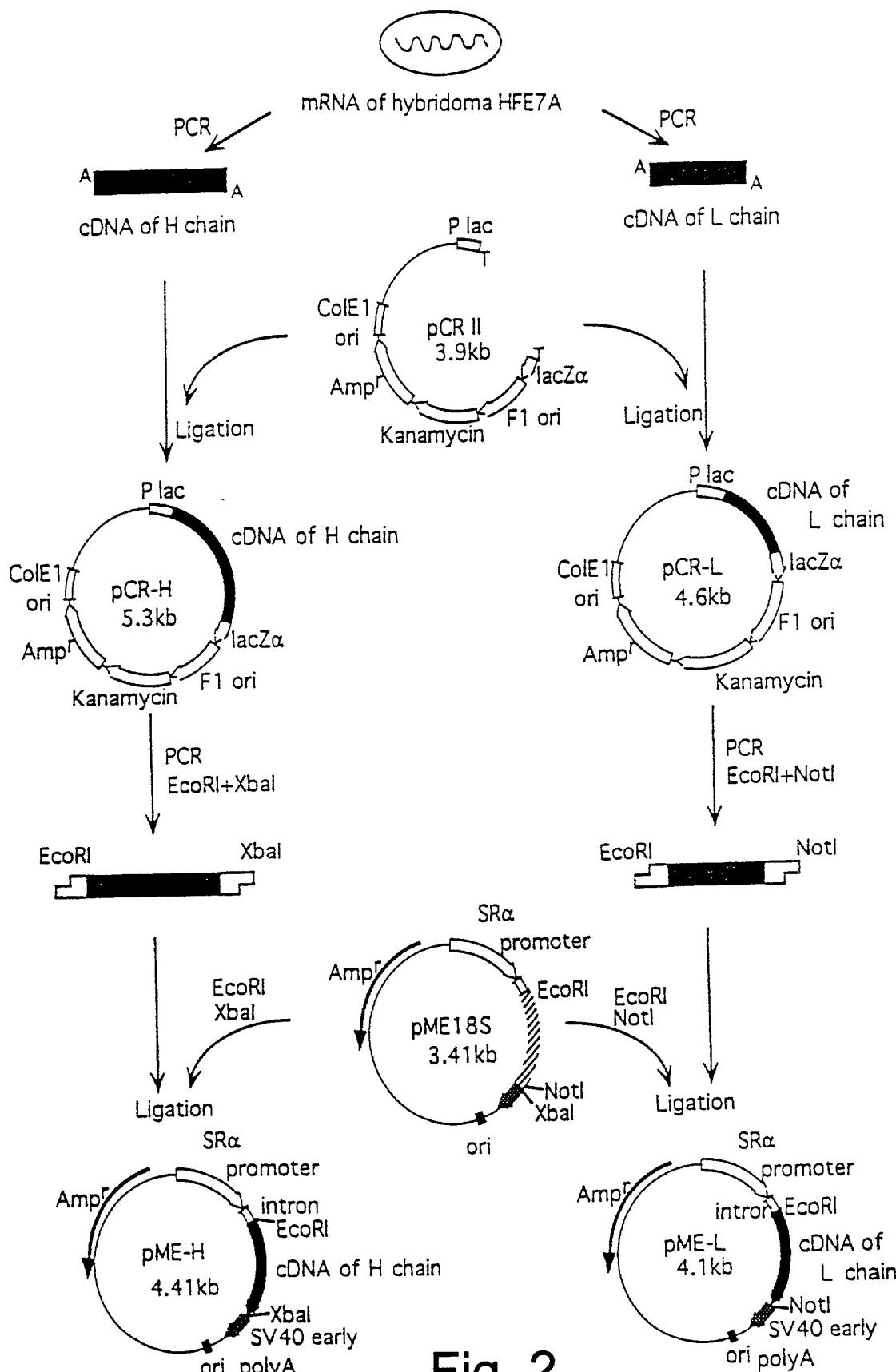


Fig. 2

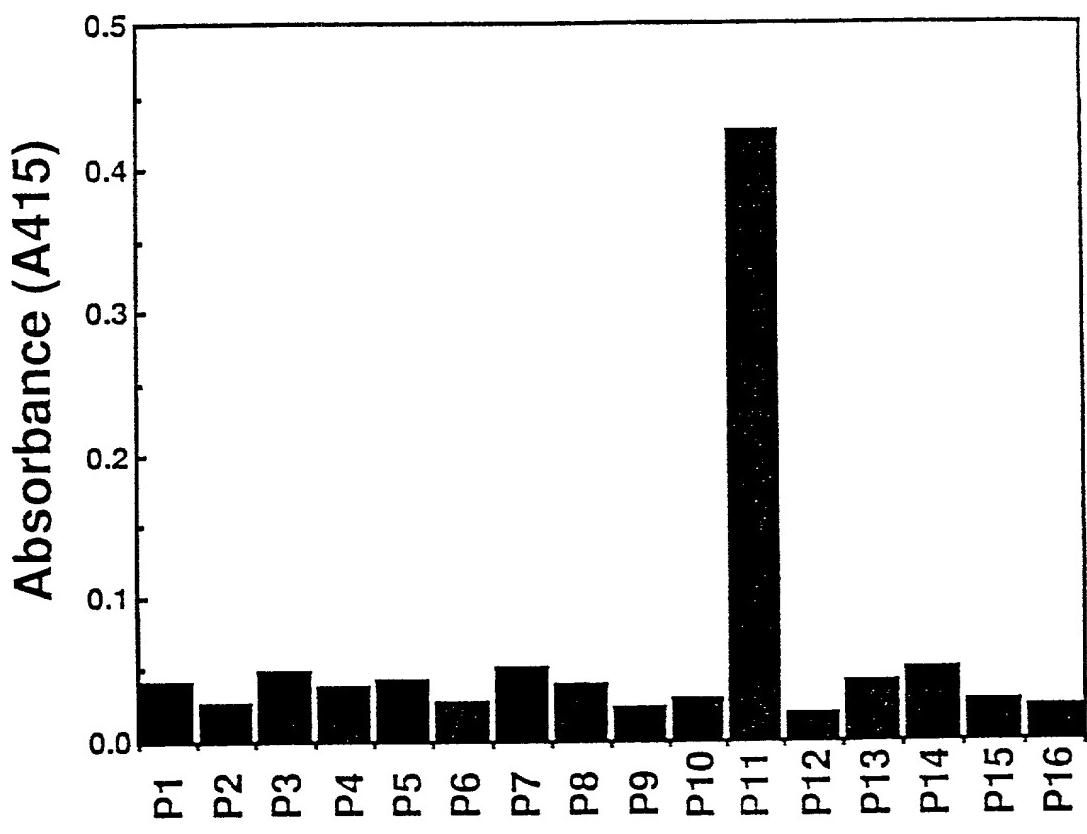


Fig. 3

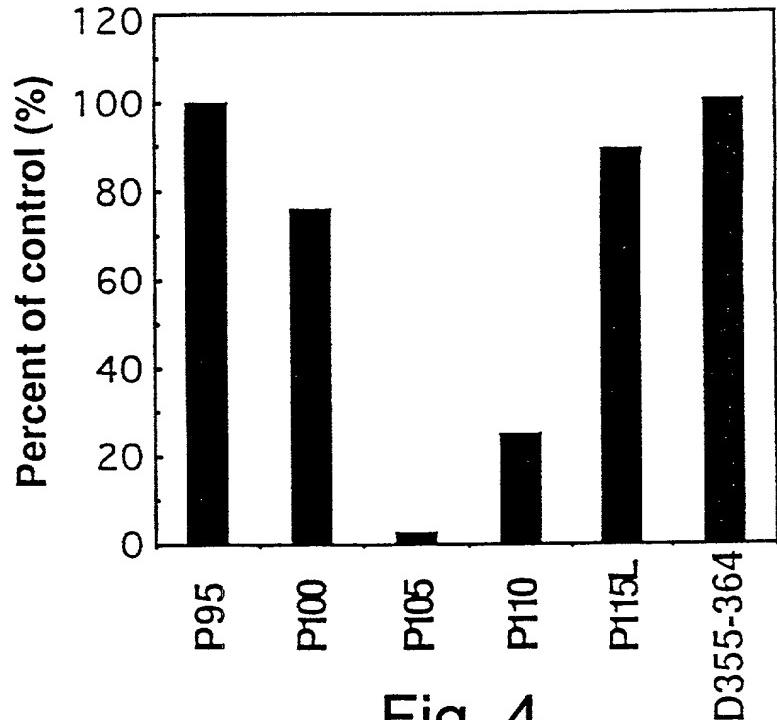


Fig. 4

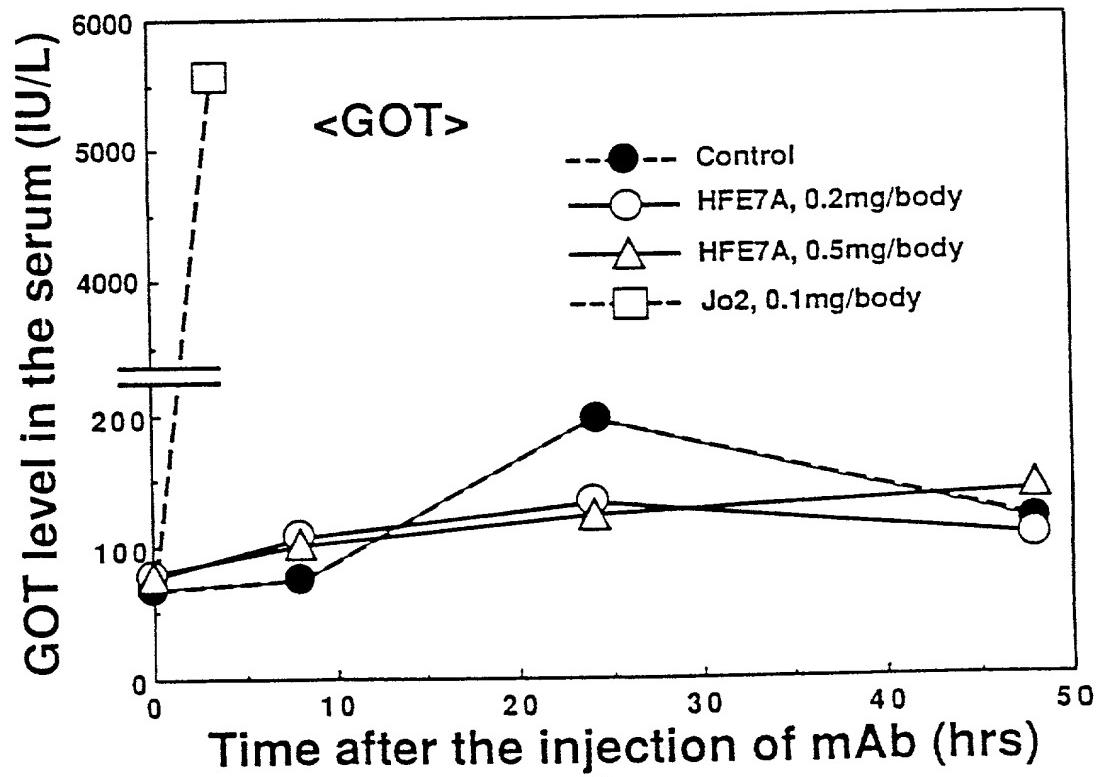
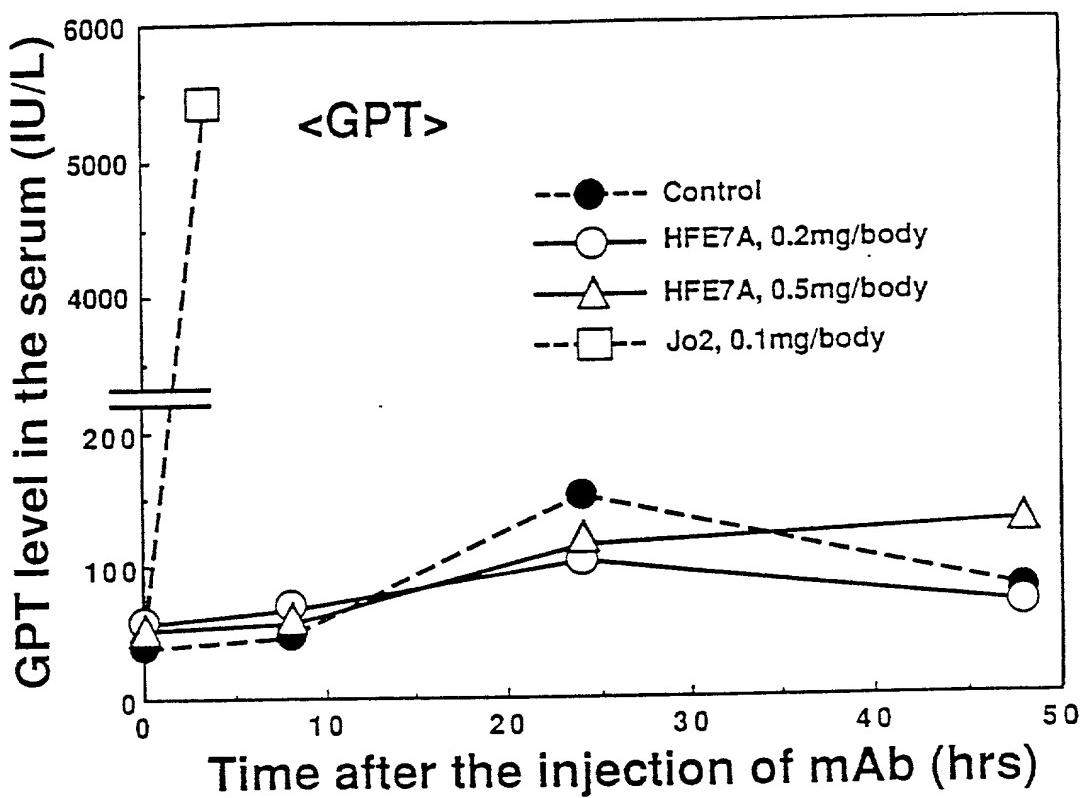


Fig. 5

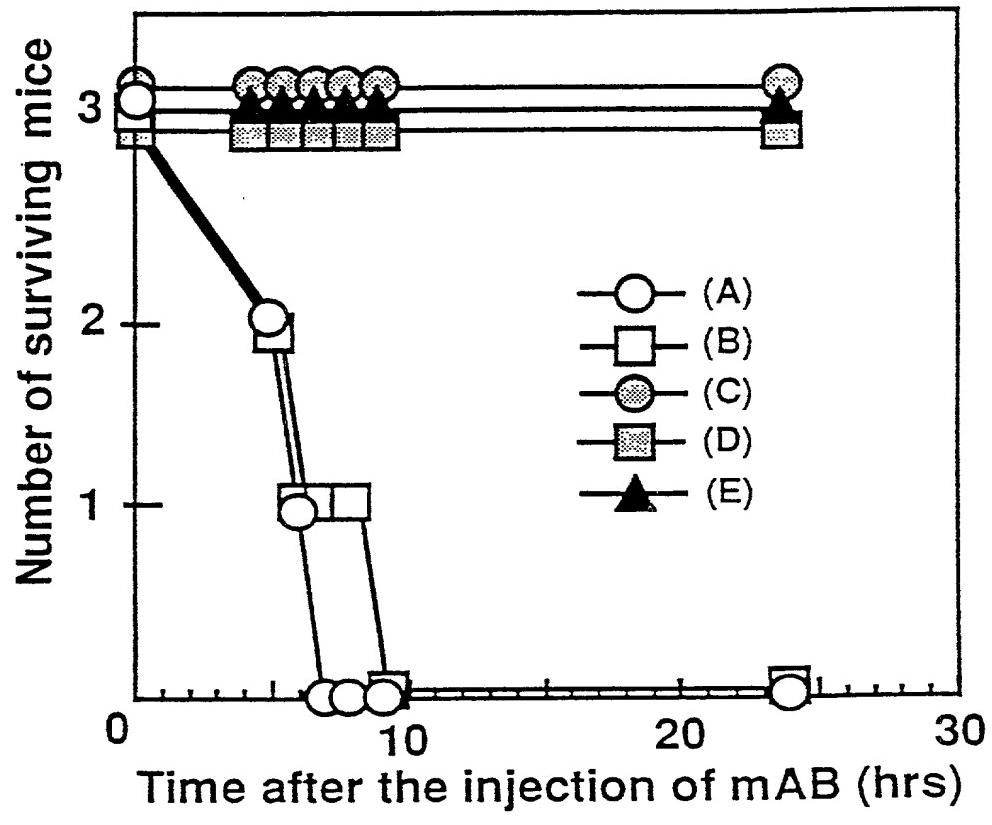
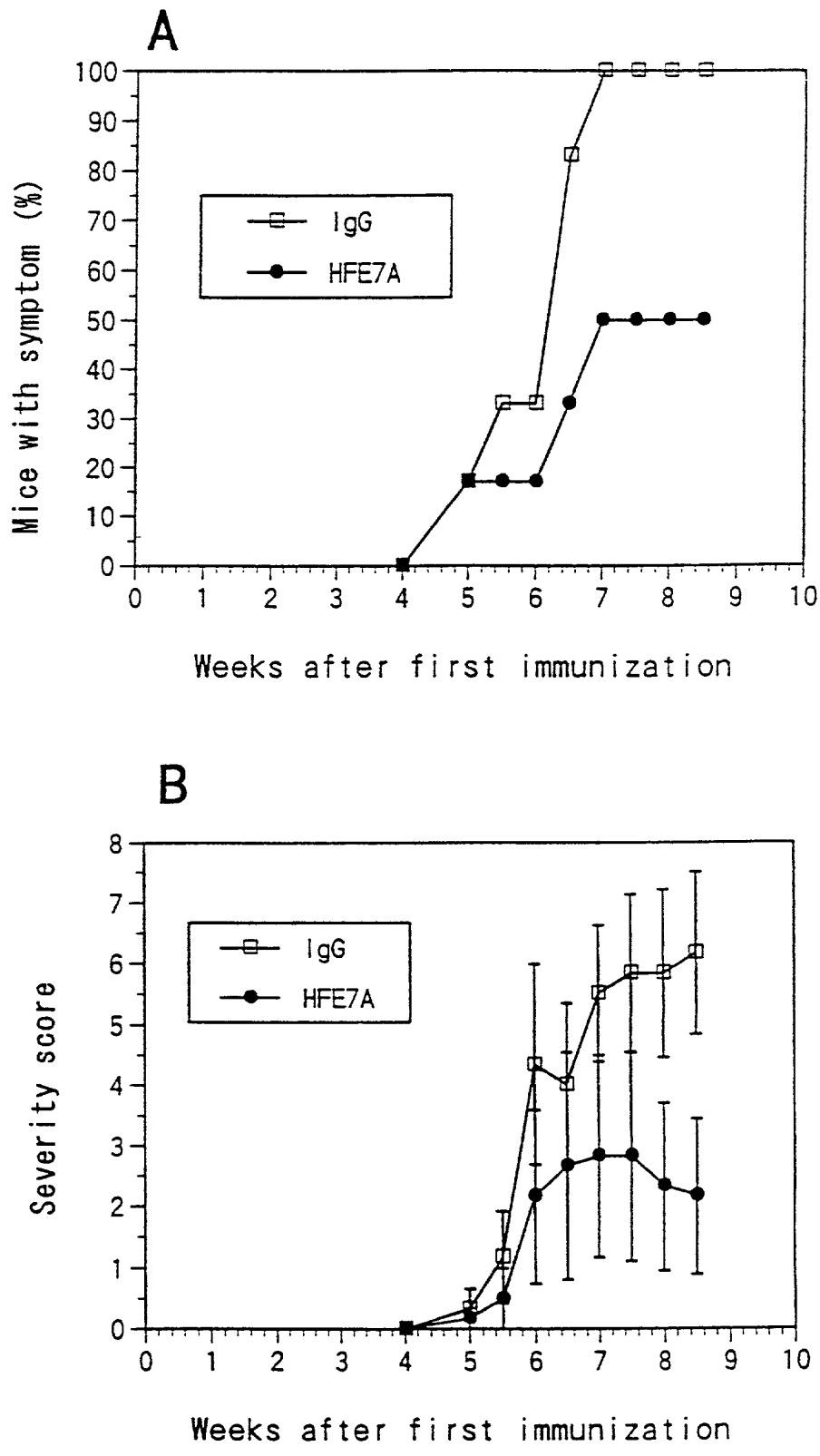


Fig. 6



**Fig. 7**

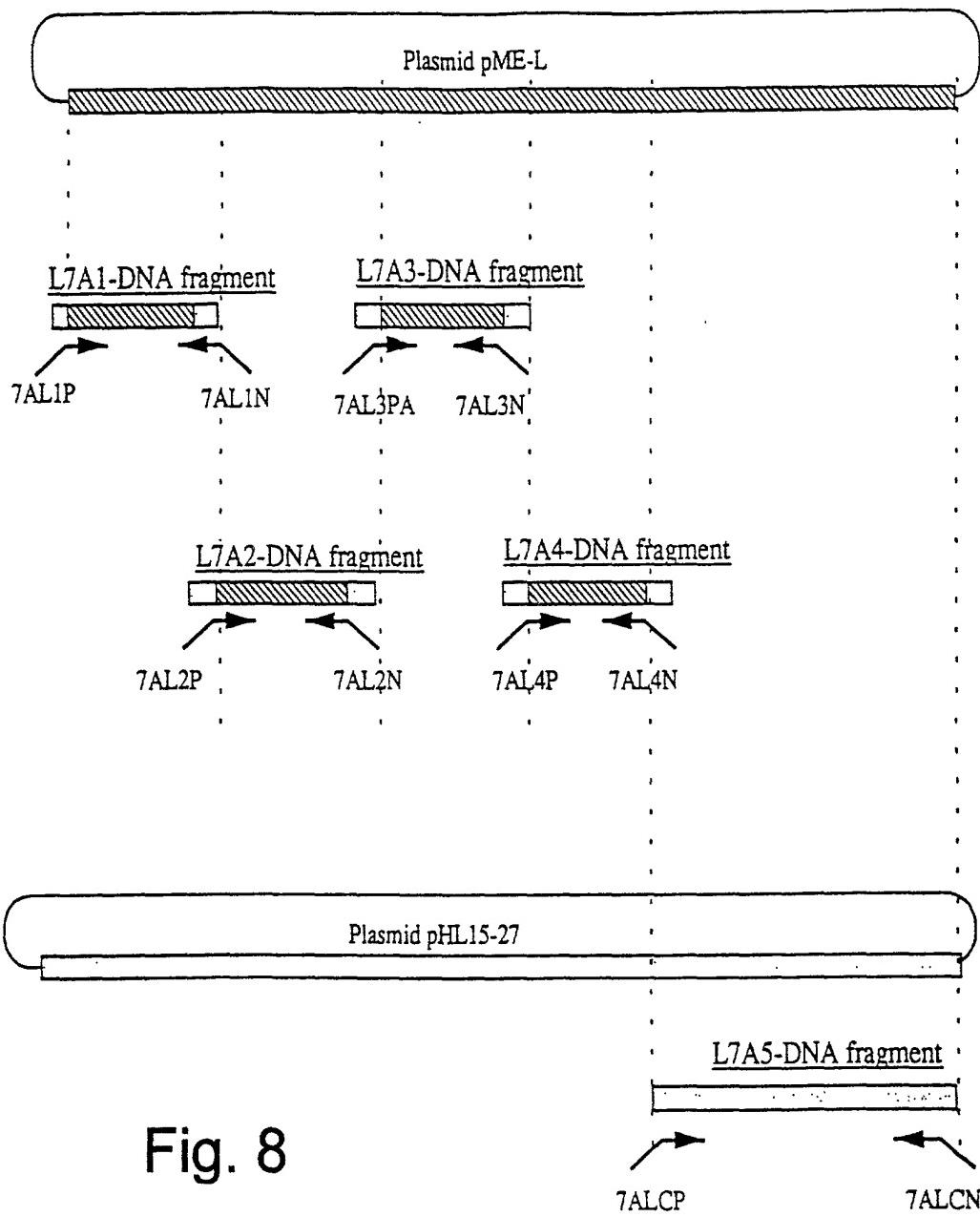
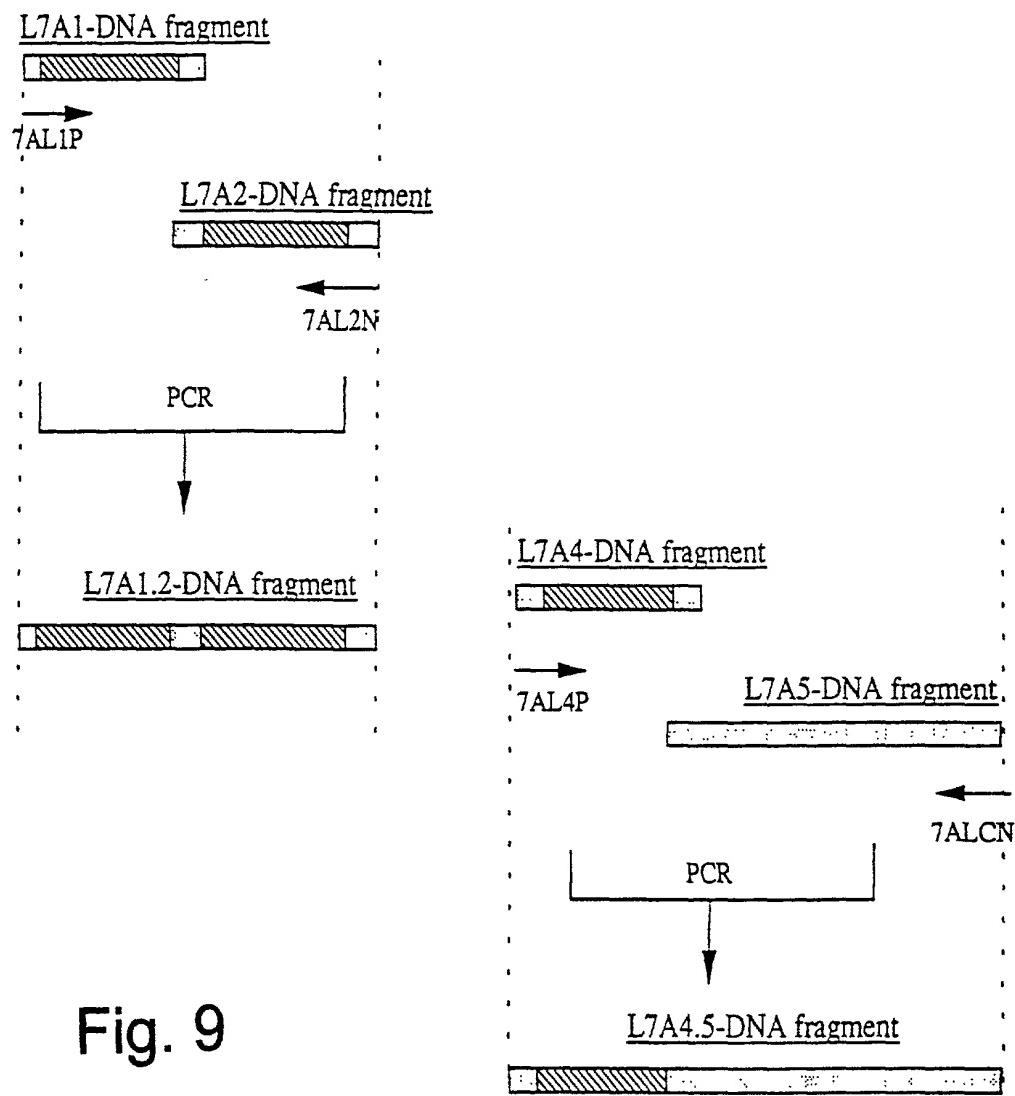


Fig. 8



**Fig. 9**

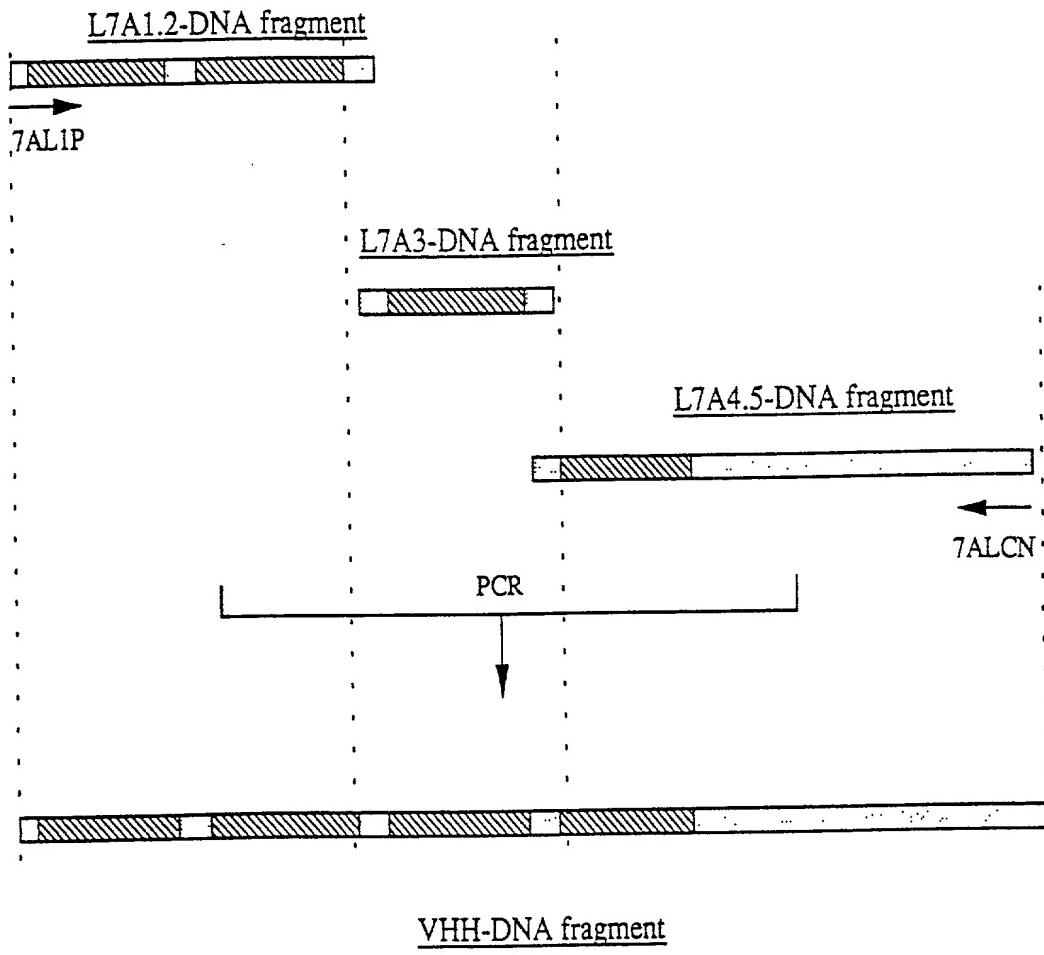
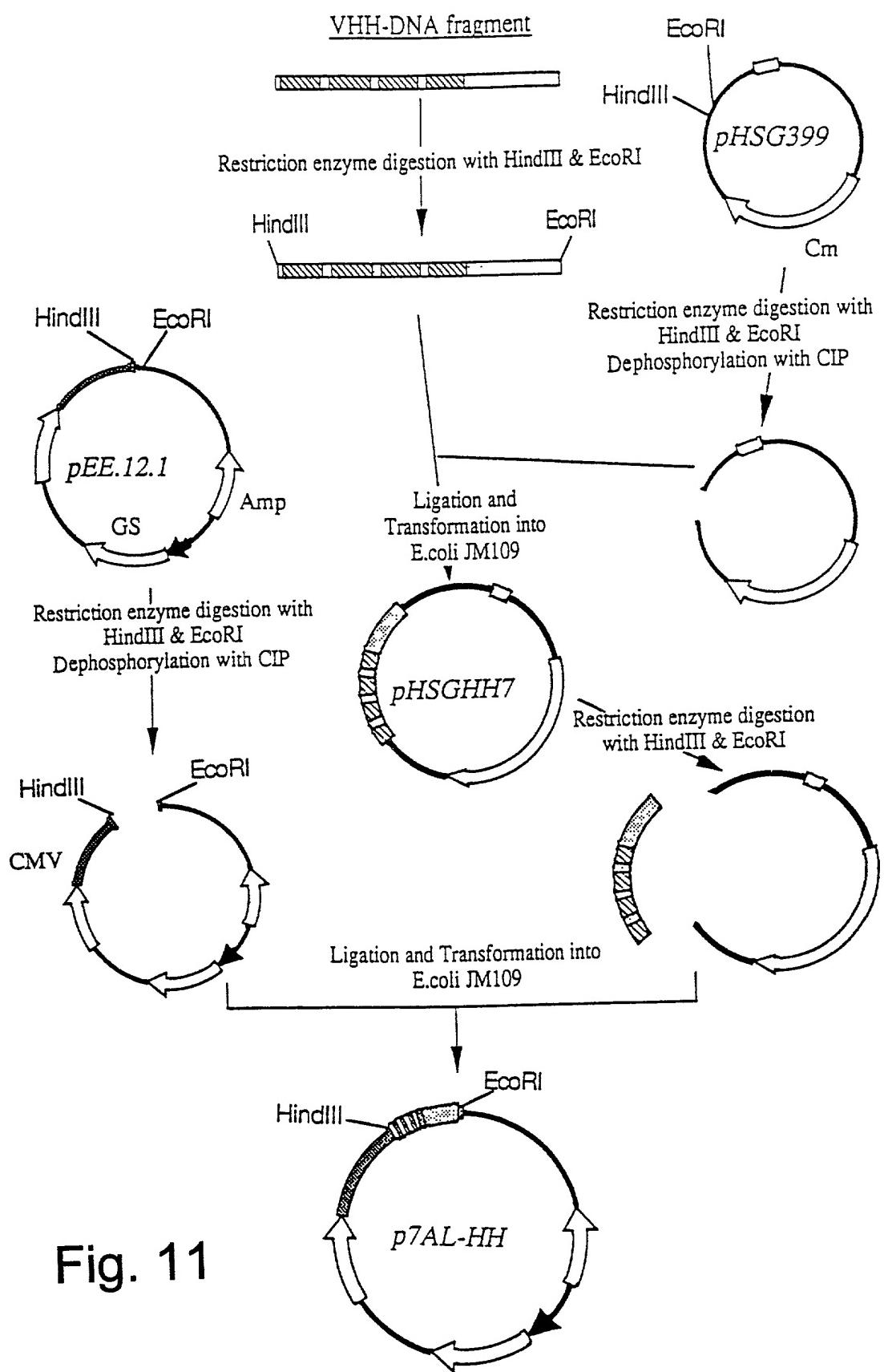
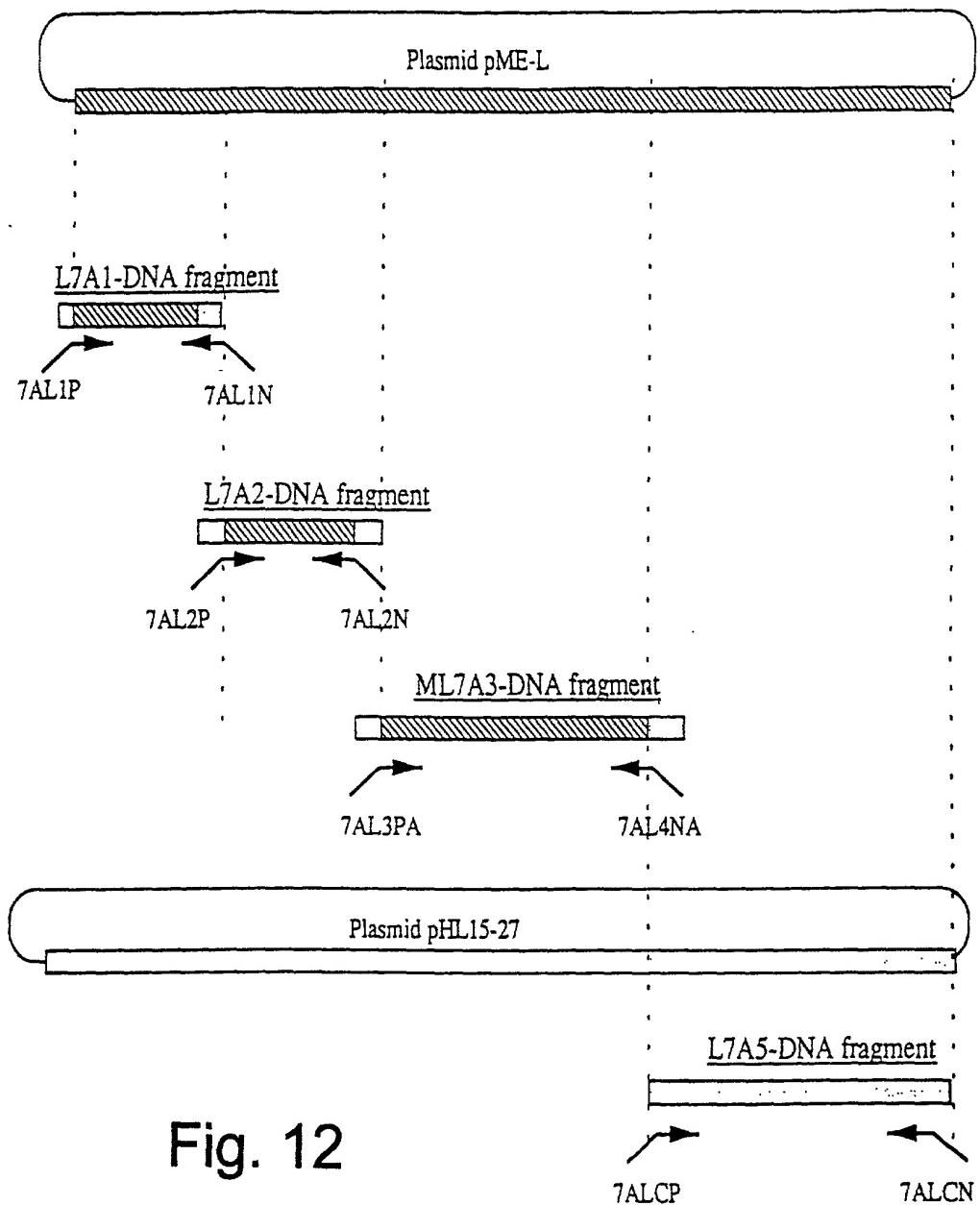
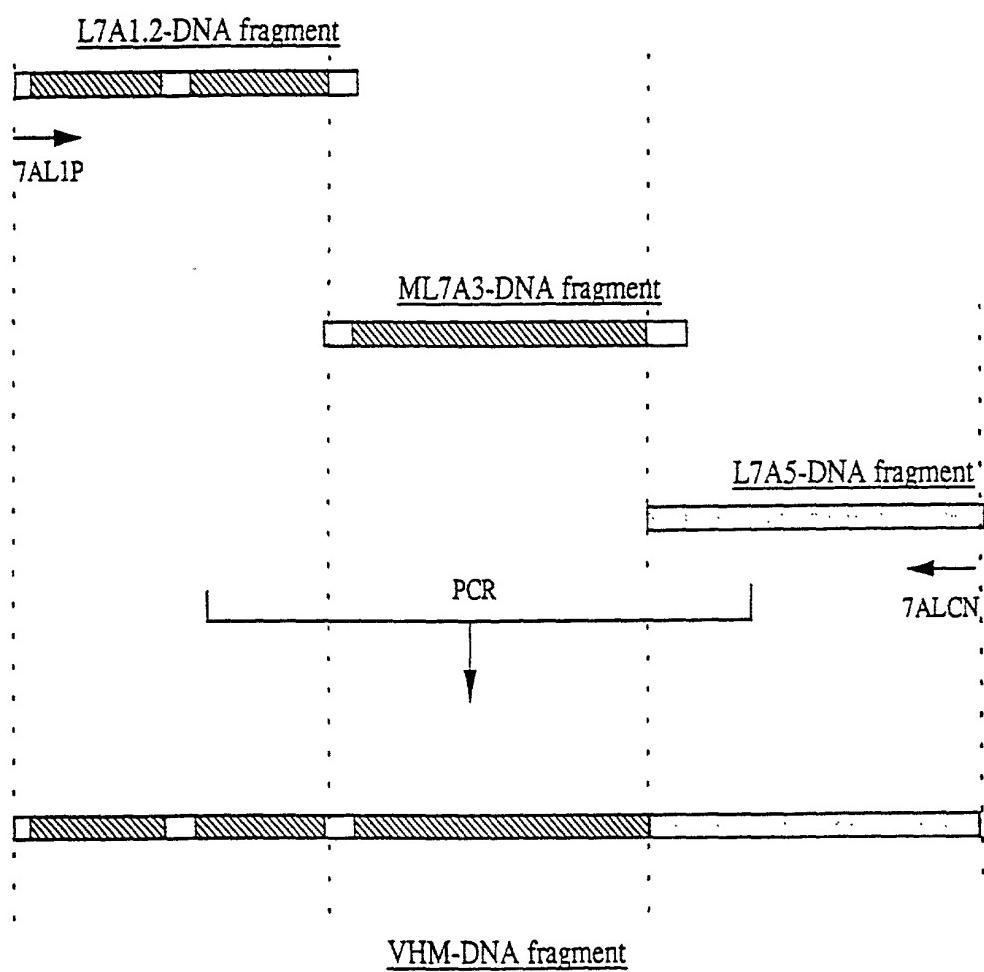


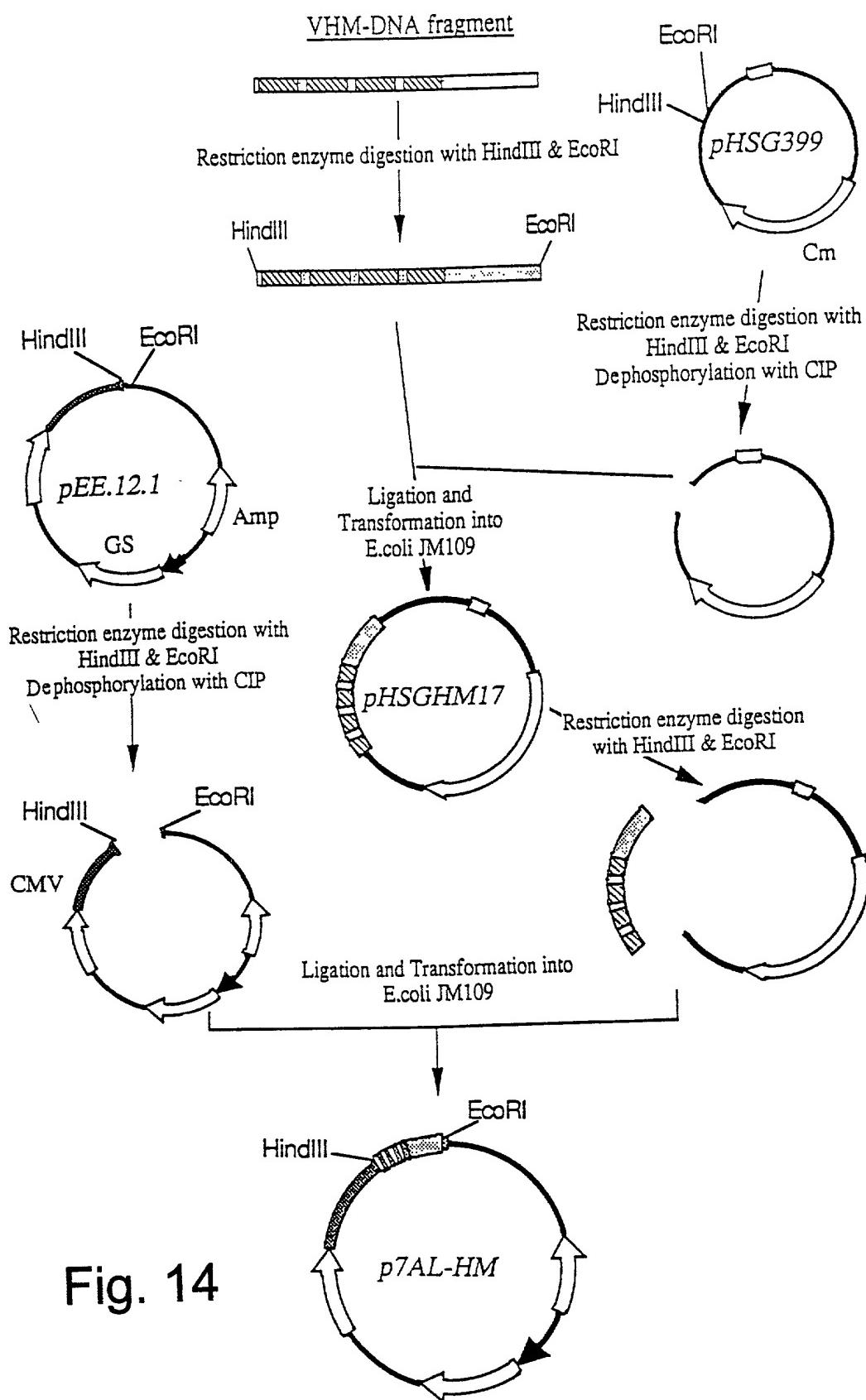
Fig. 10

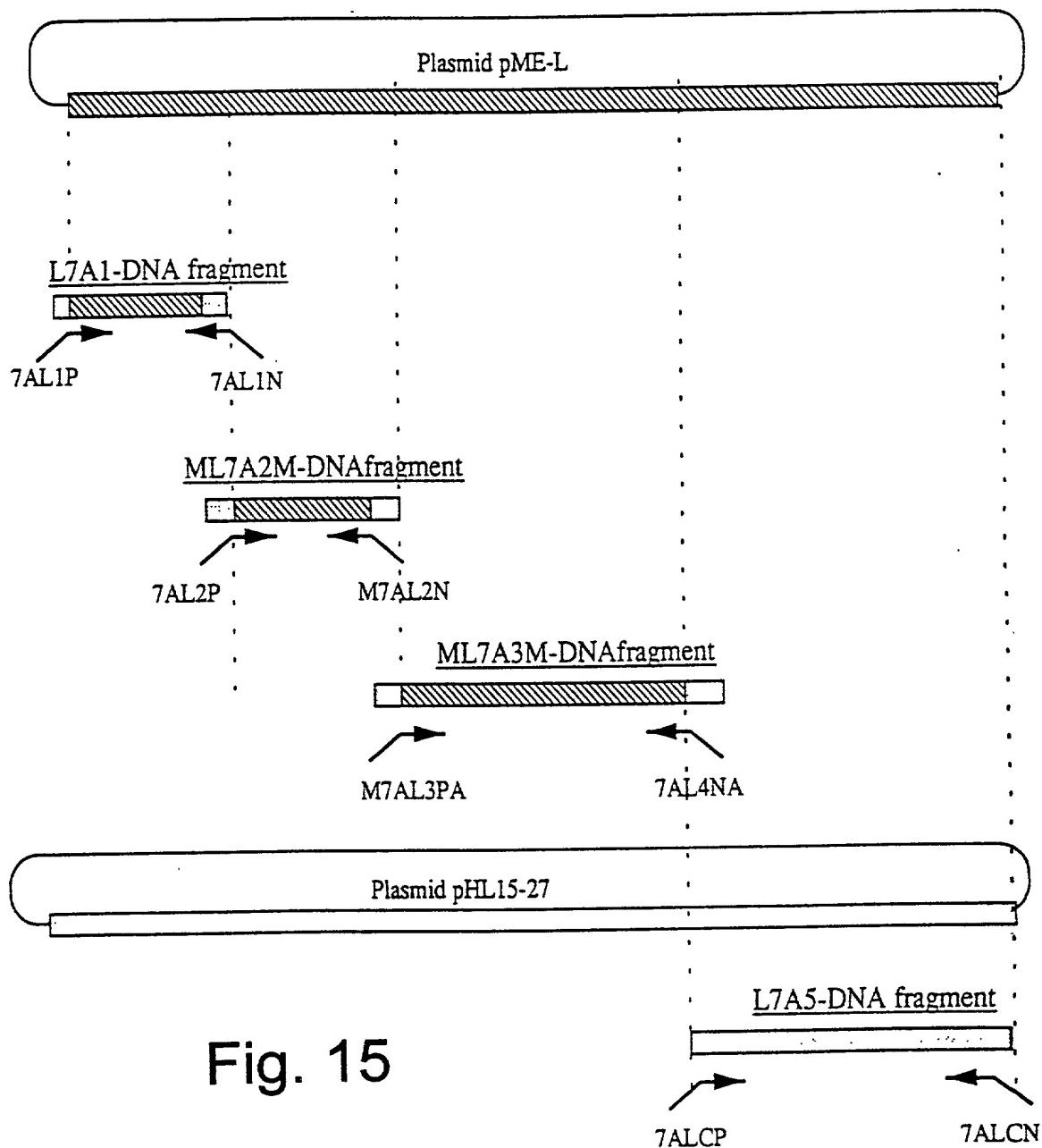


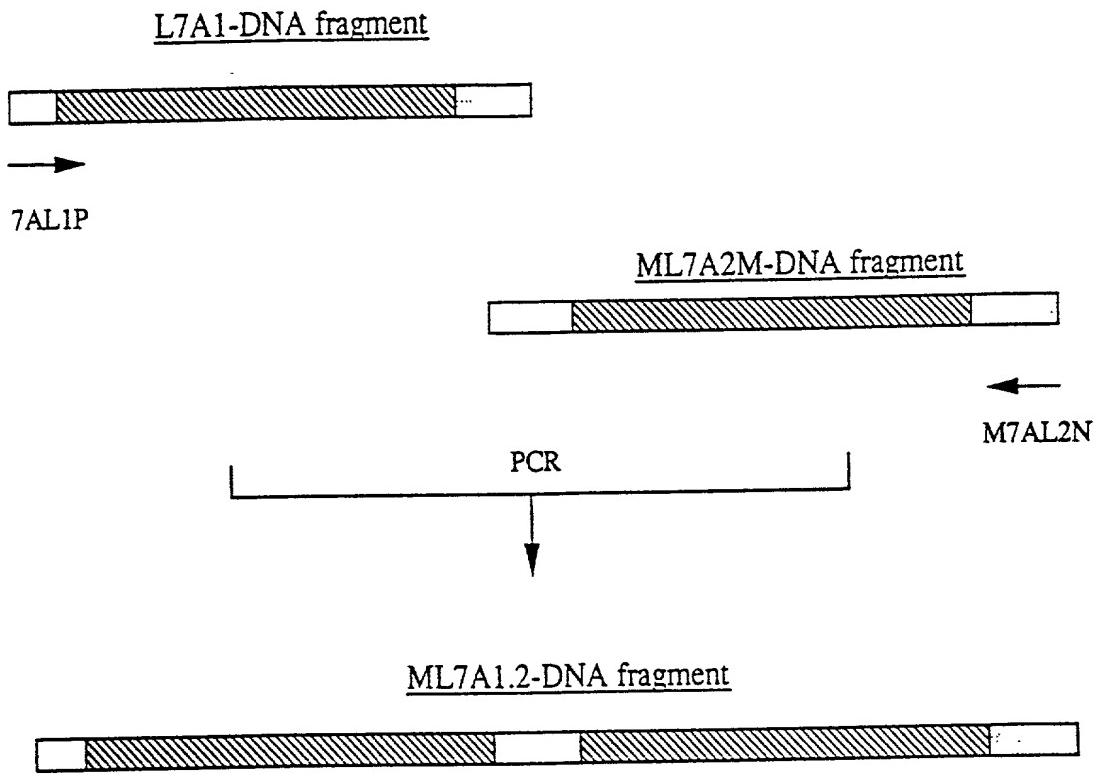




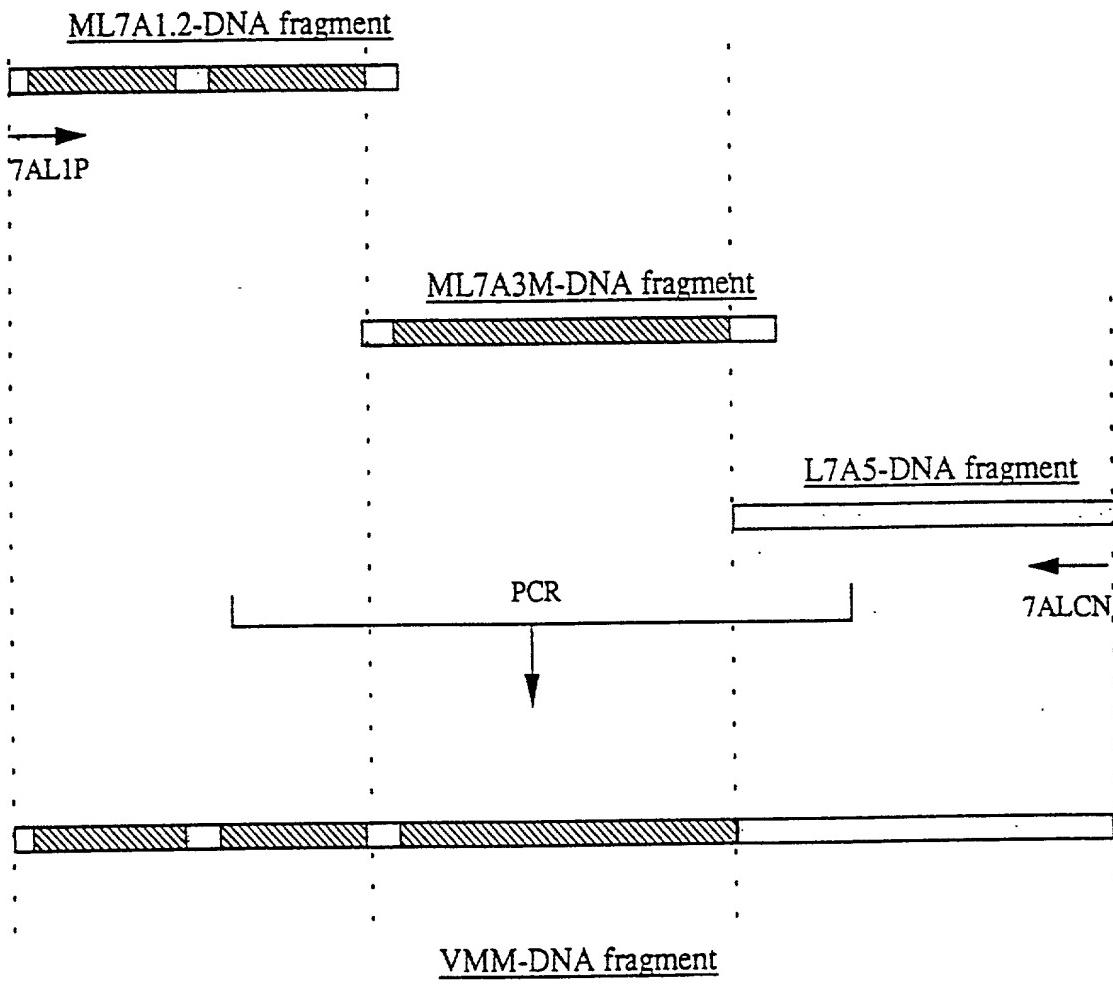
**Fig. 13**



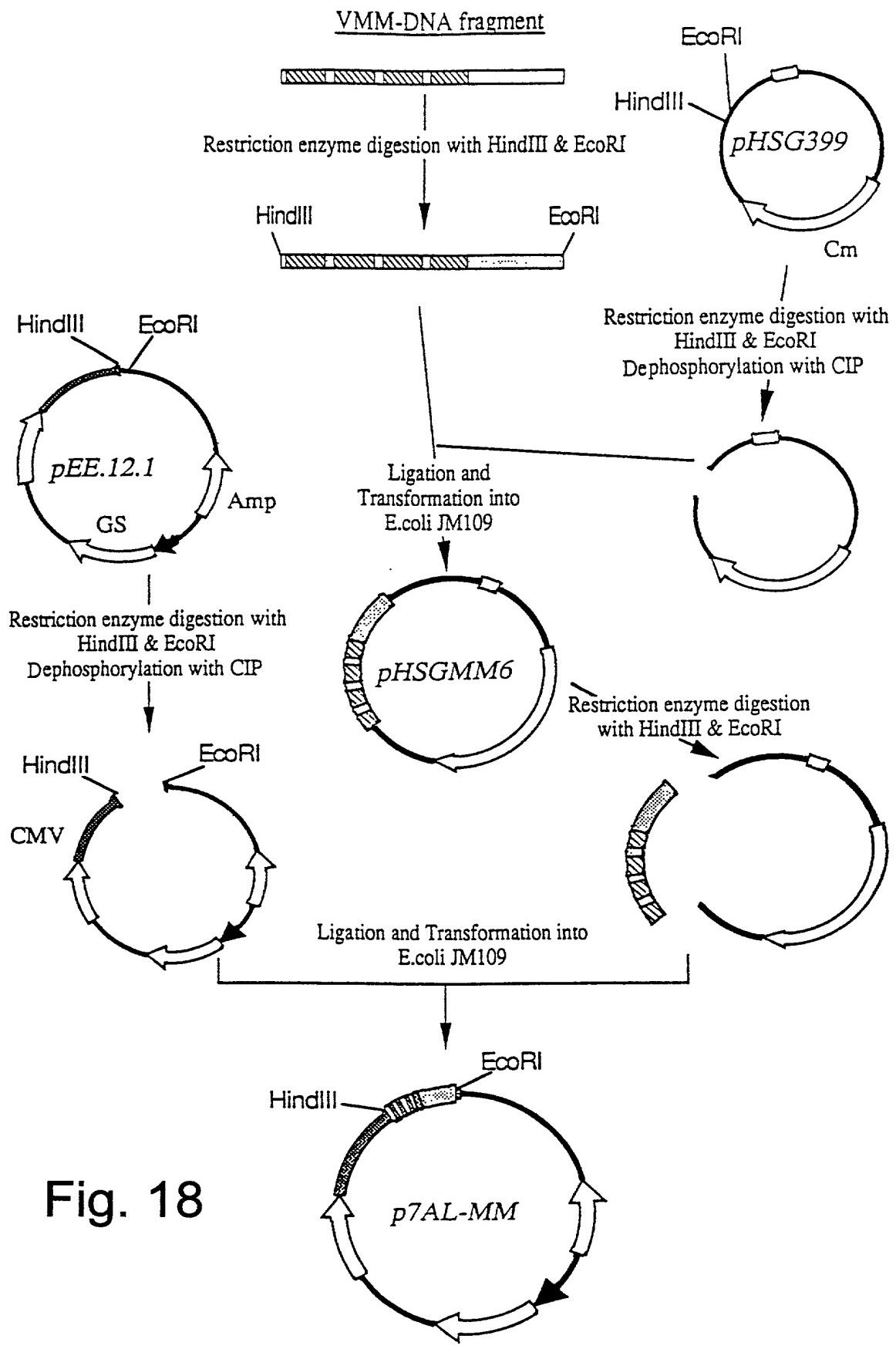




**Fig. 16**



**Fig. 17**



**Fig. 18**

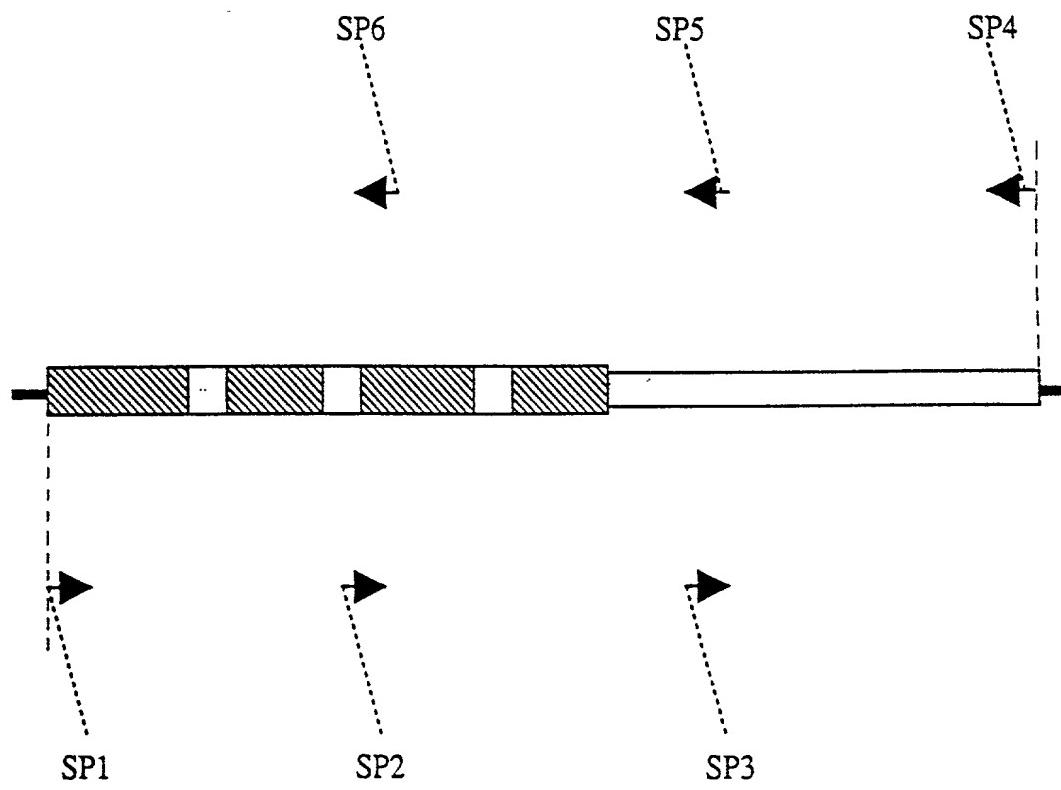


Fig. 19

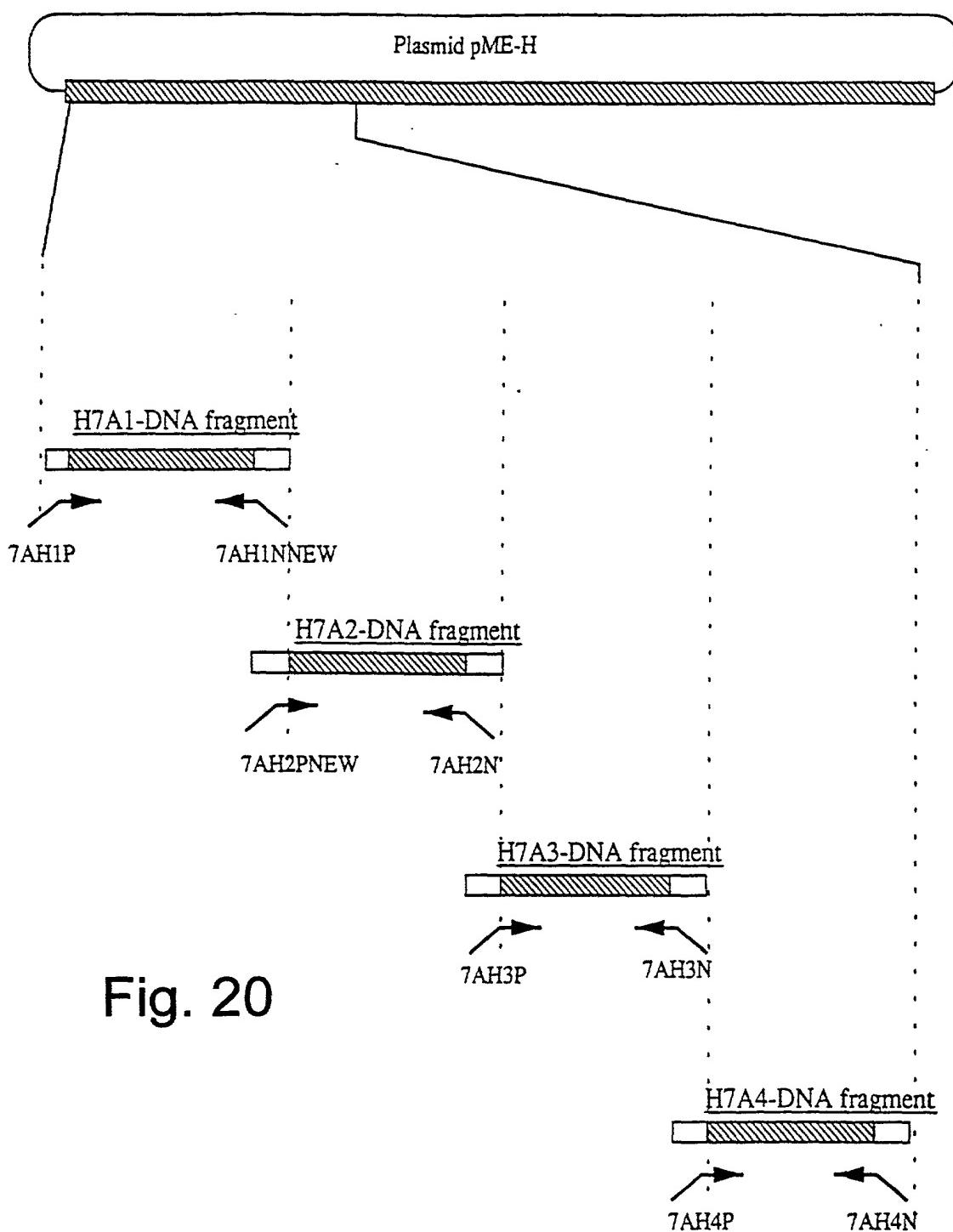


Fig. 20

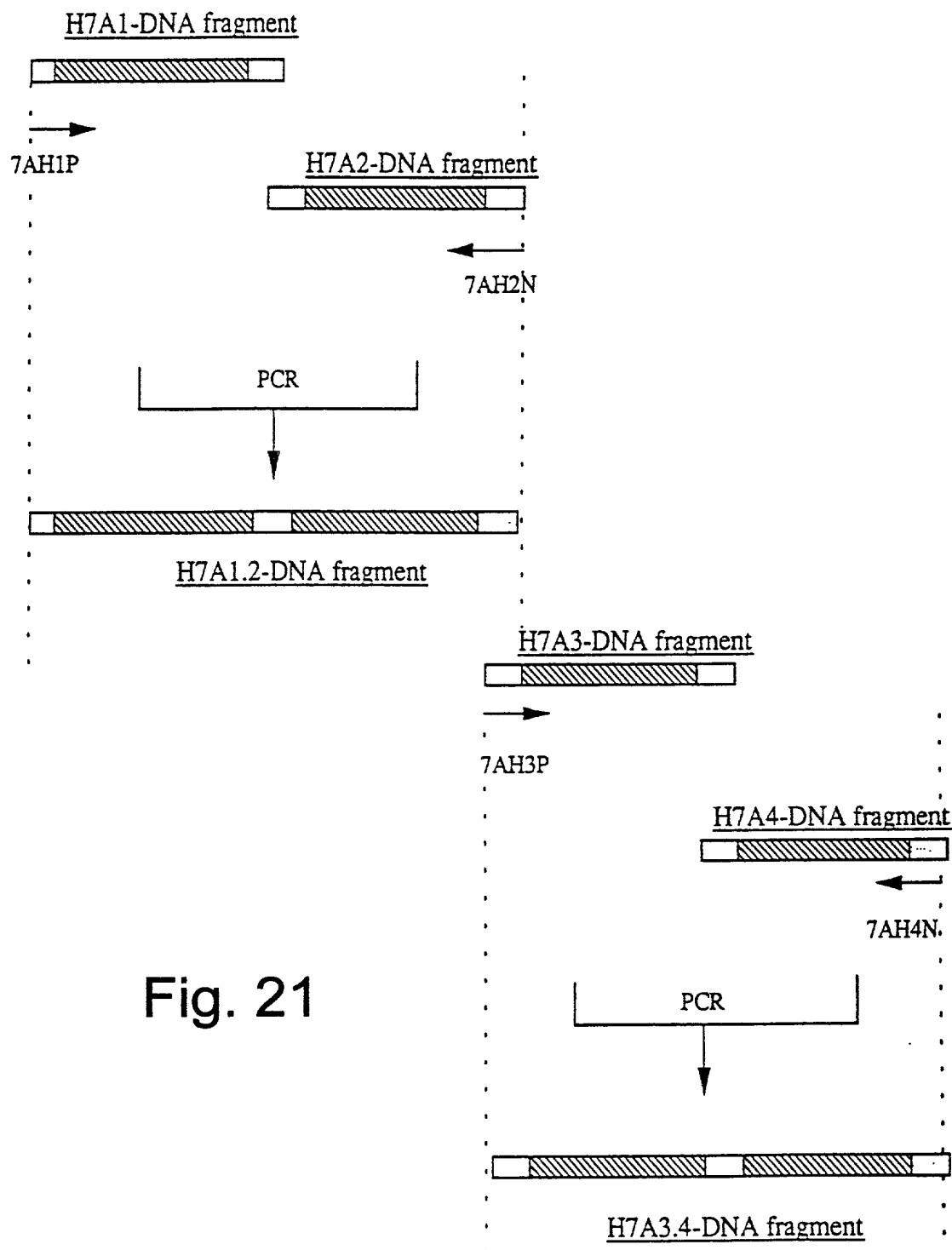


Fig. 21

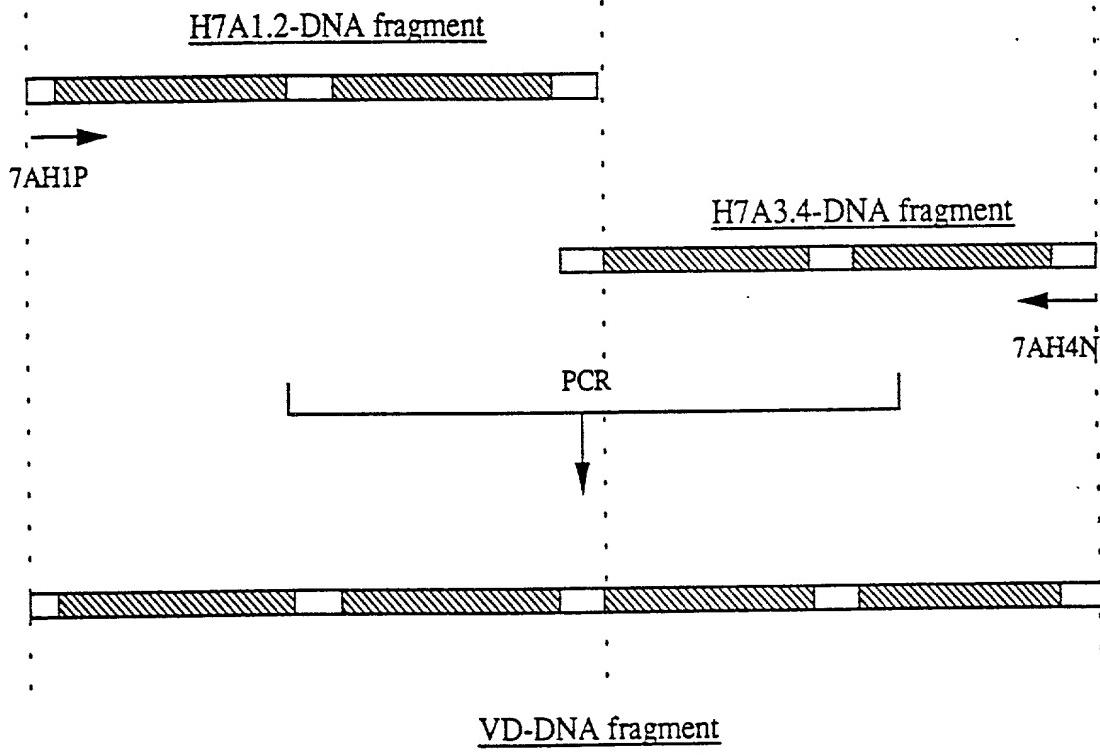


Fig. 22

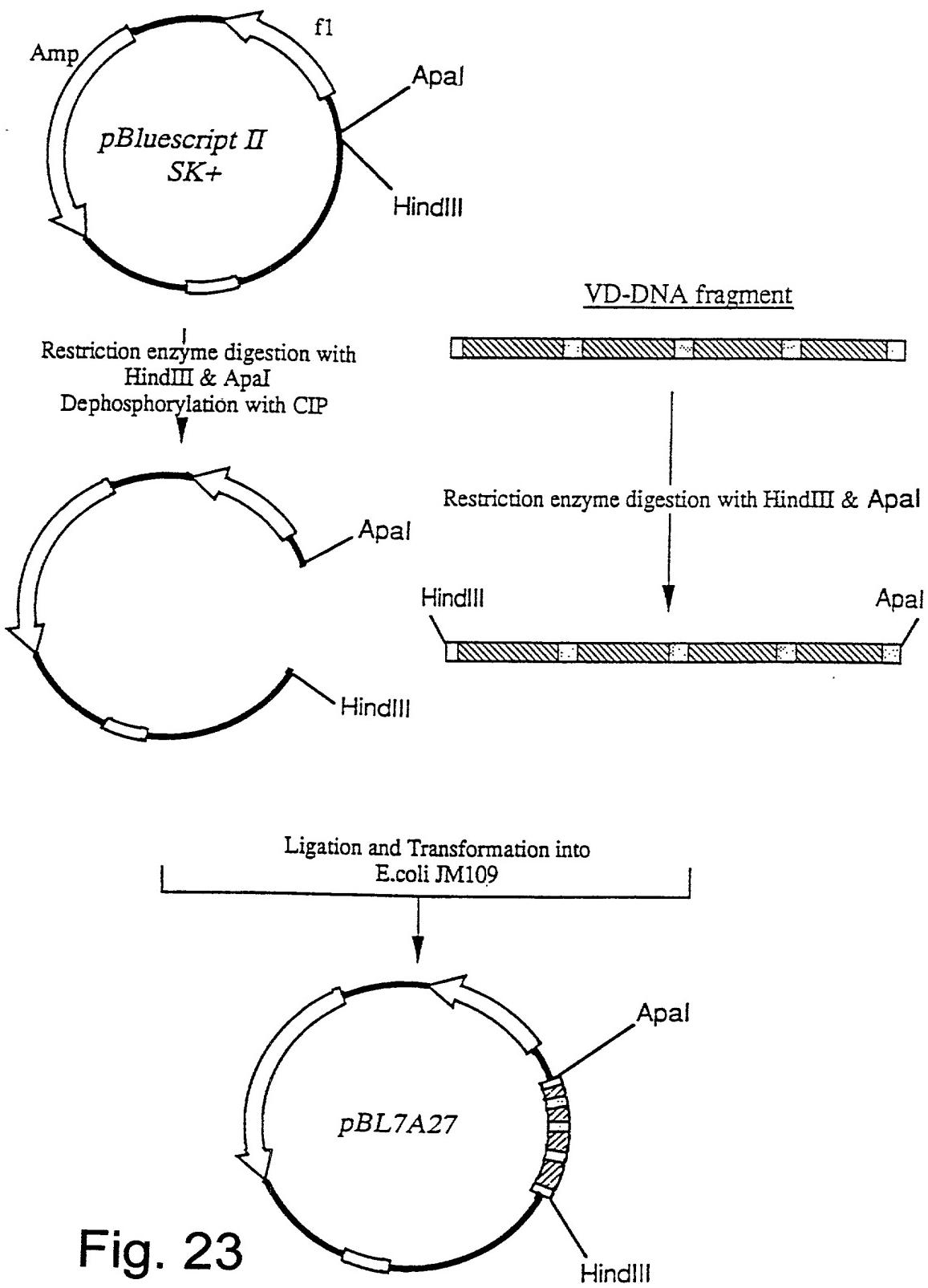


Fig. 23

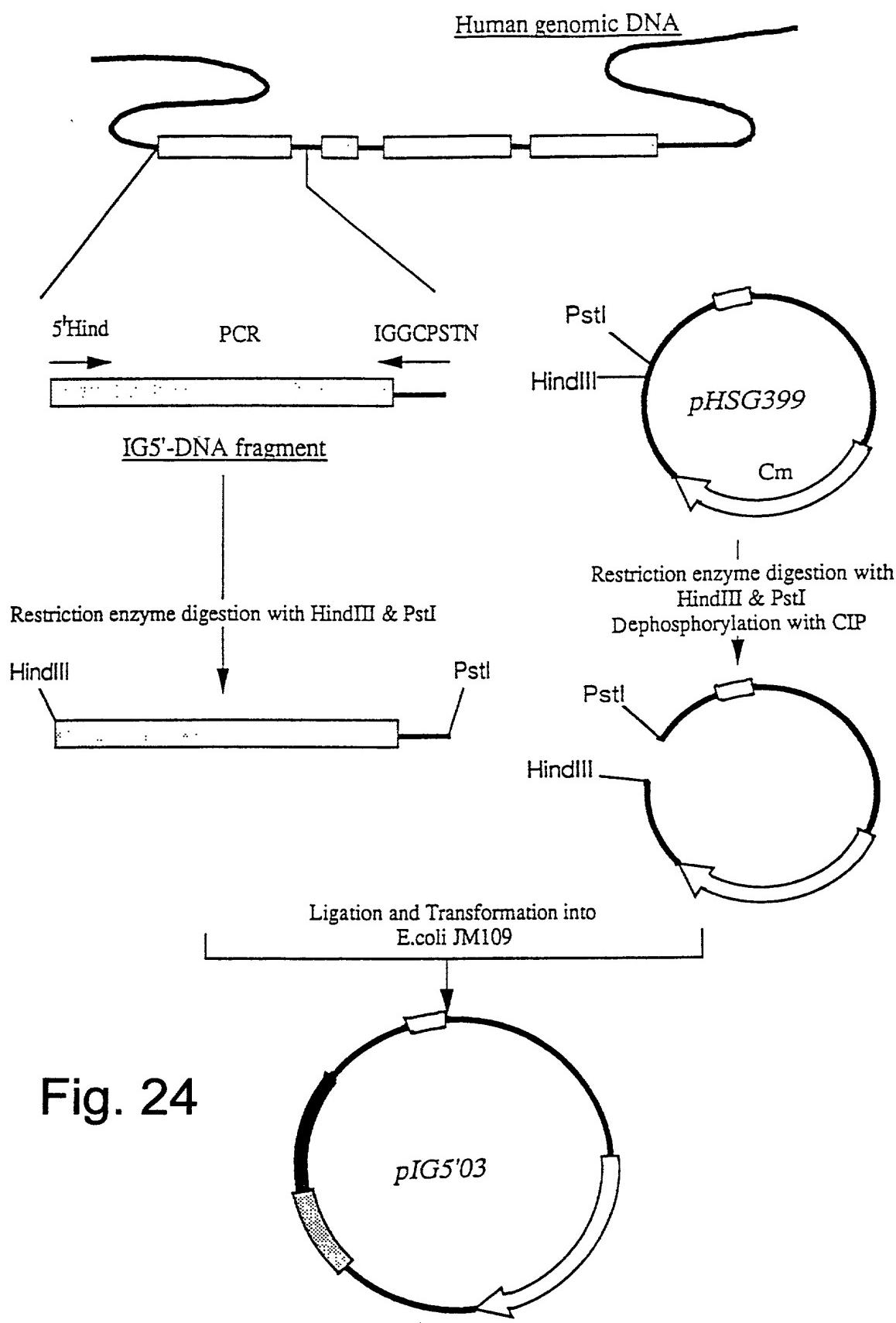
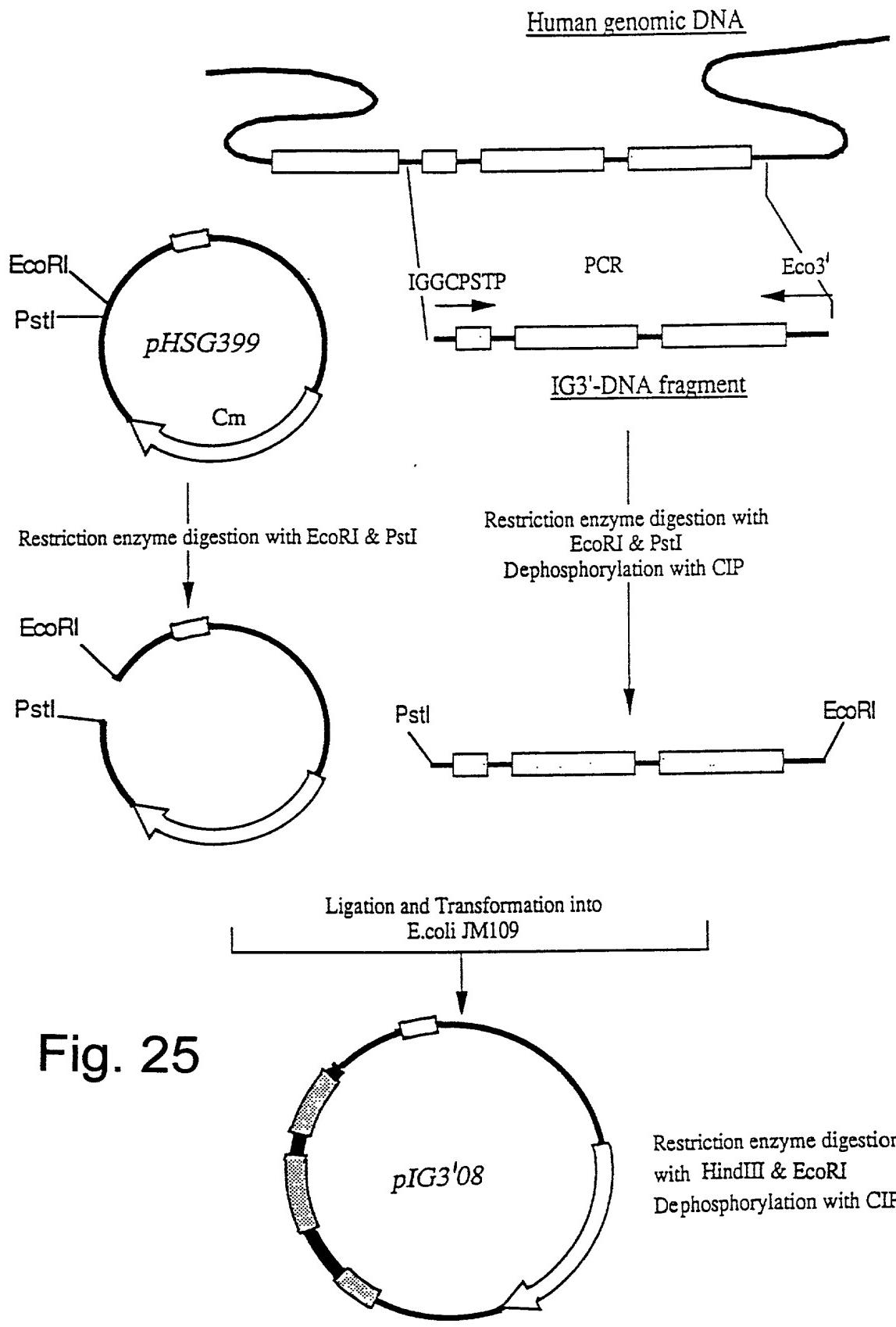
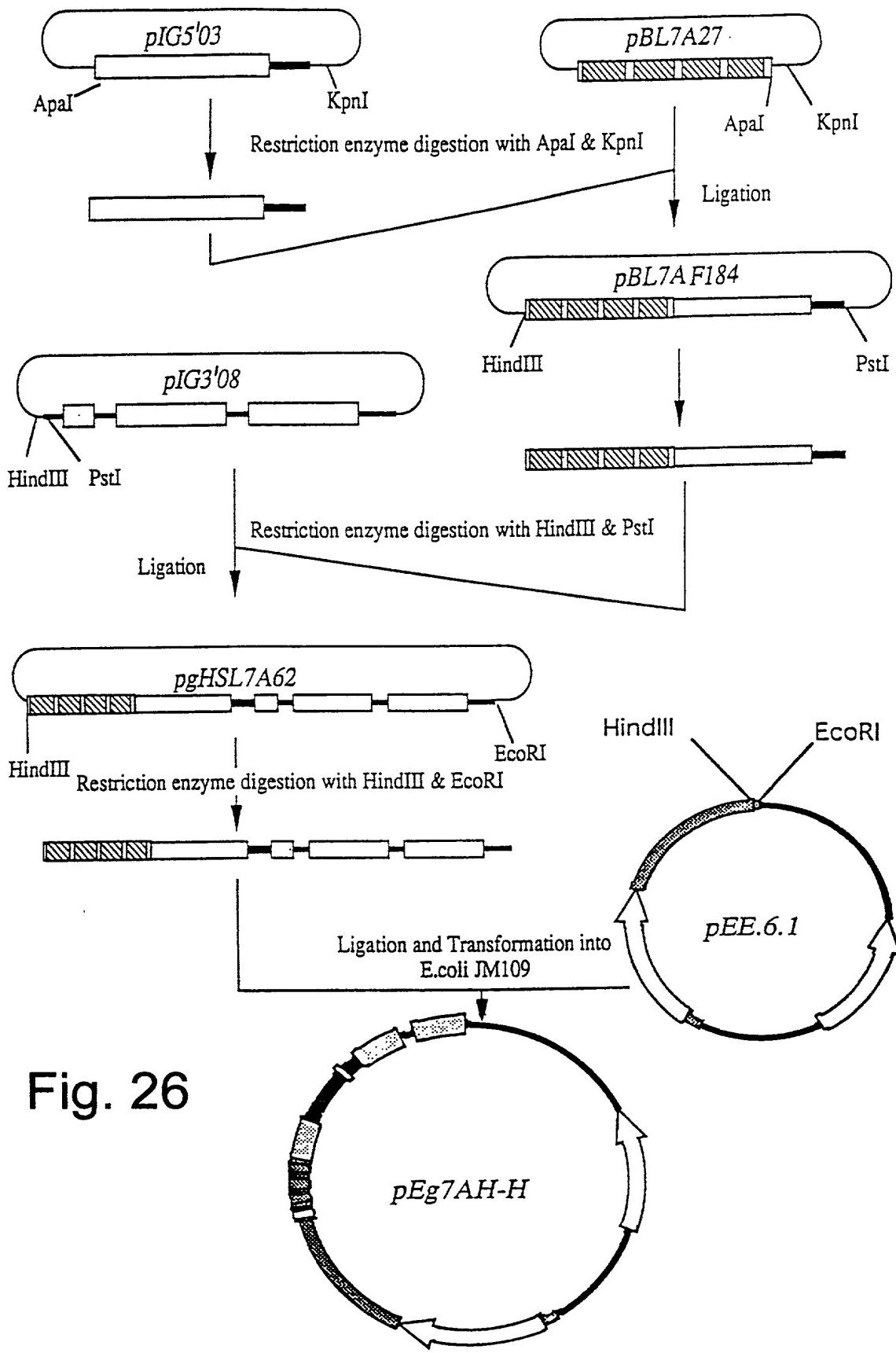


Fig. 24





**Fig. 26**

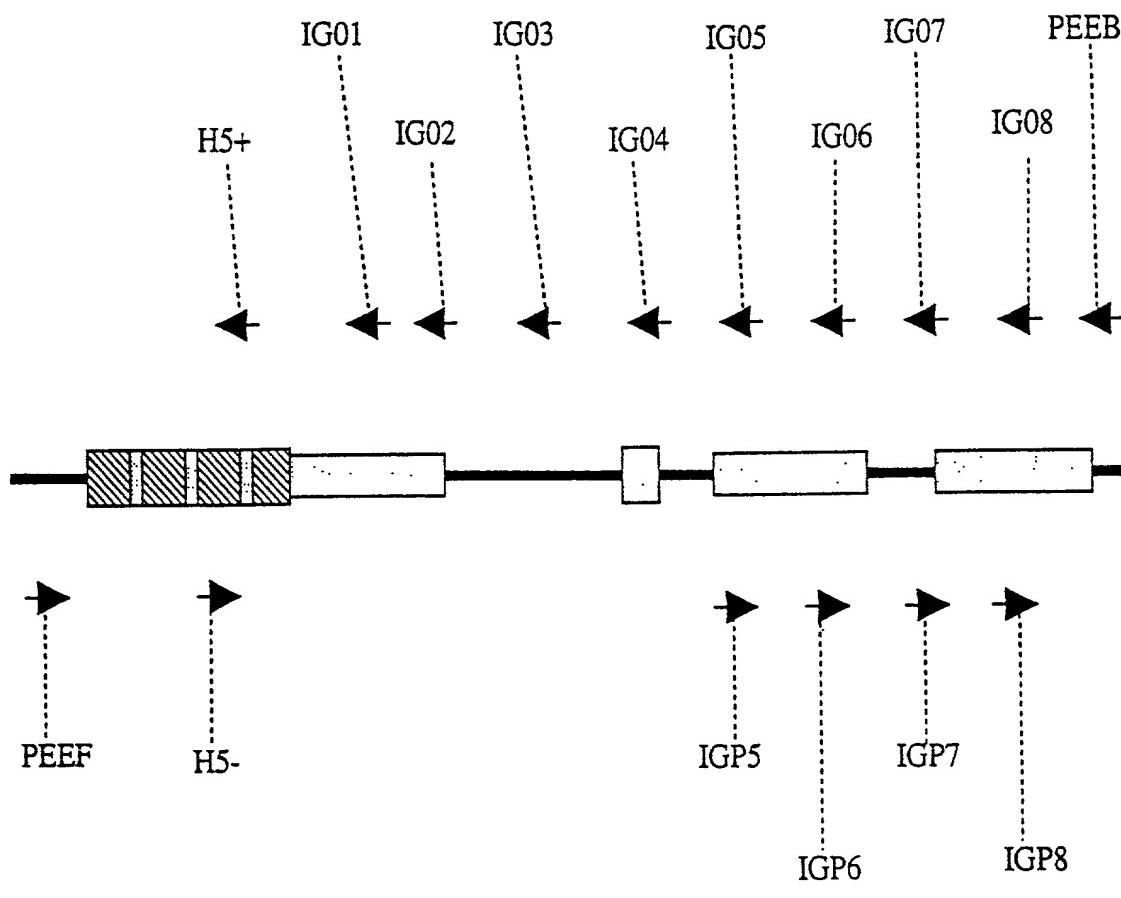


Fig. 27

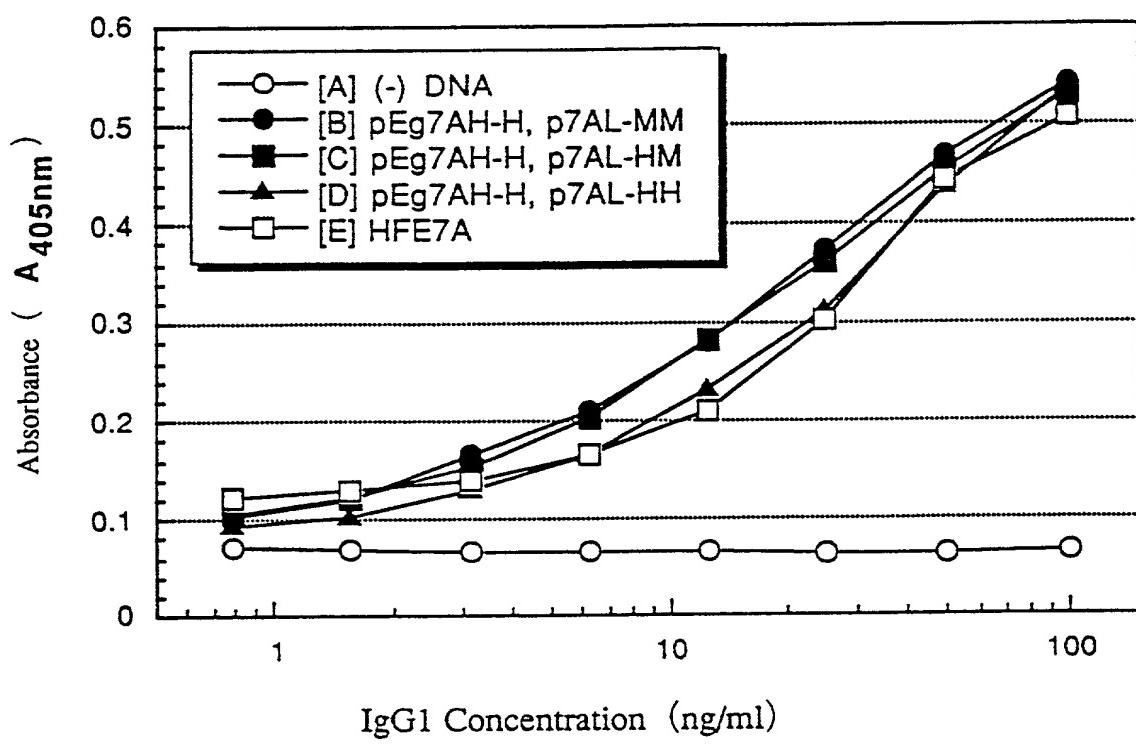


Fig. 28

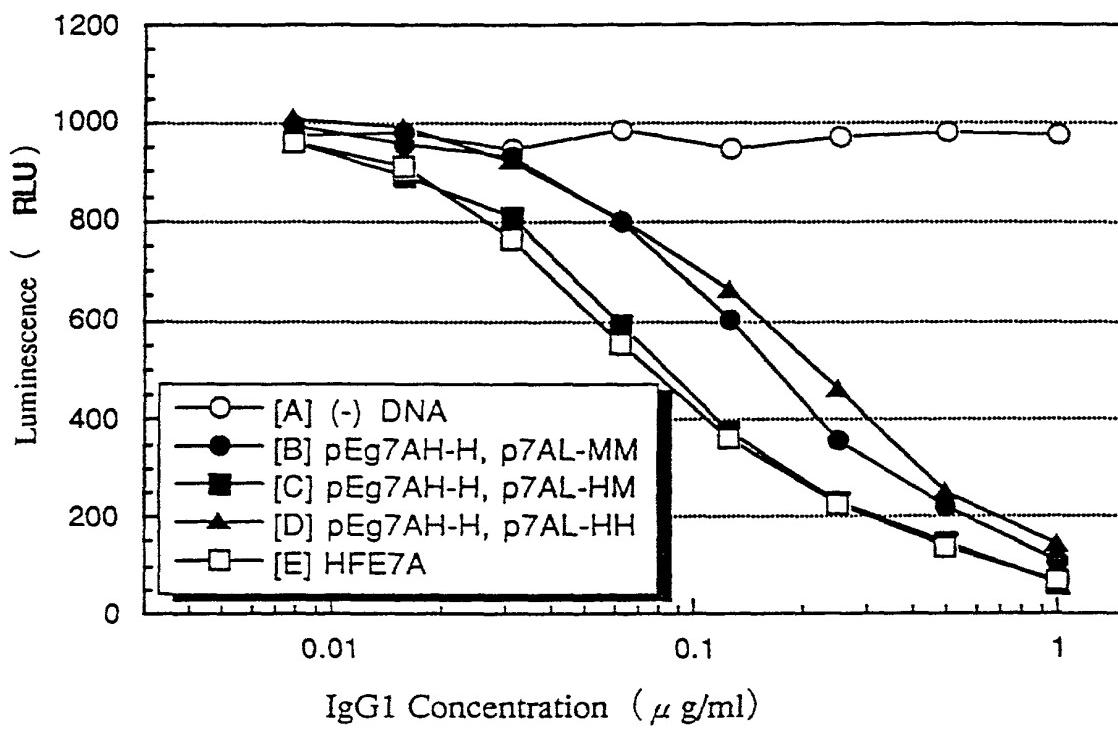


Fig. 29

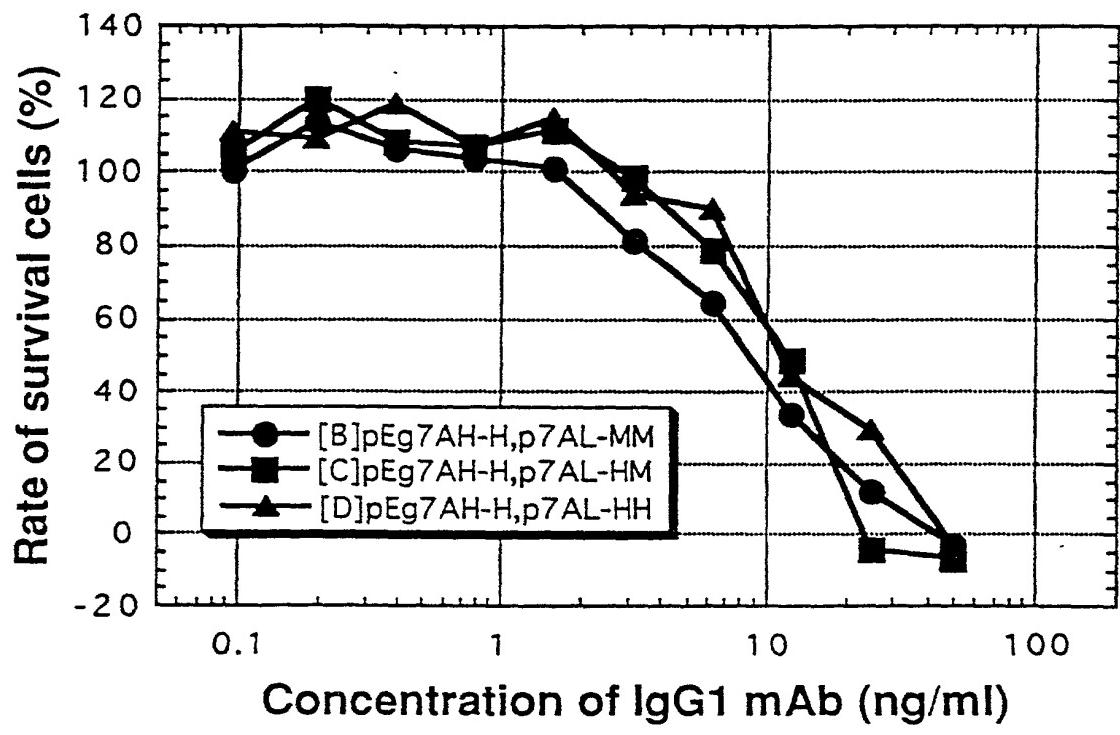


Fig. 30

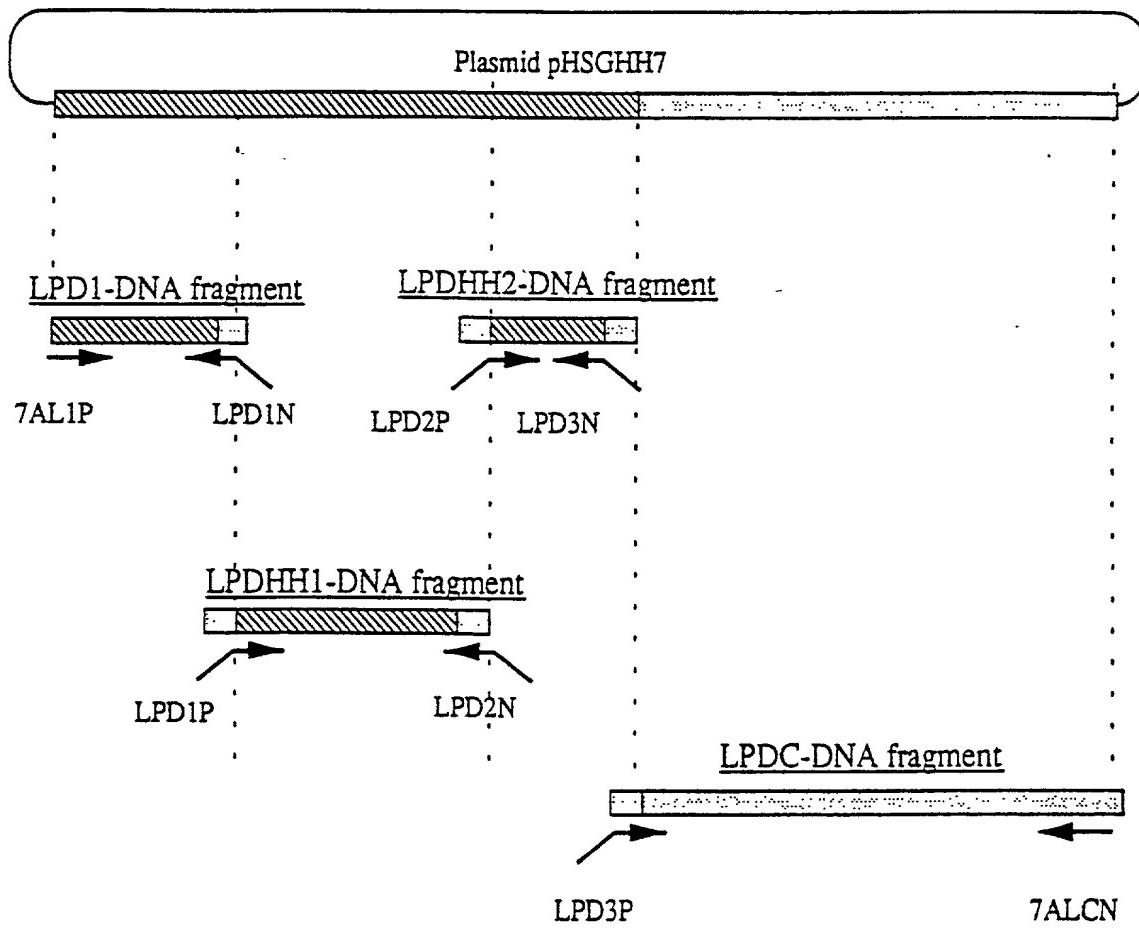


Fig. 31

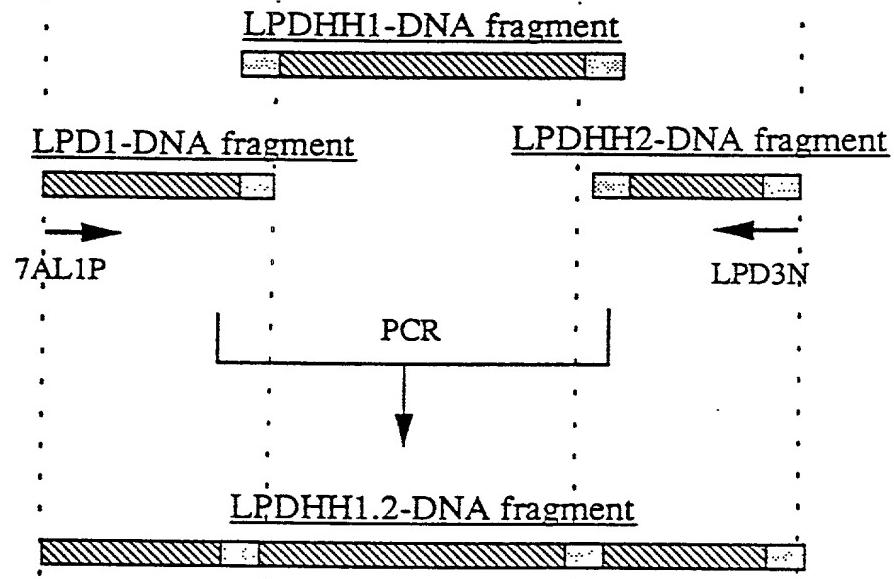


Fig. 32

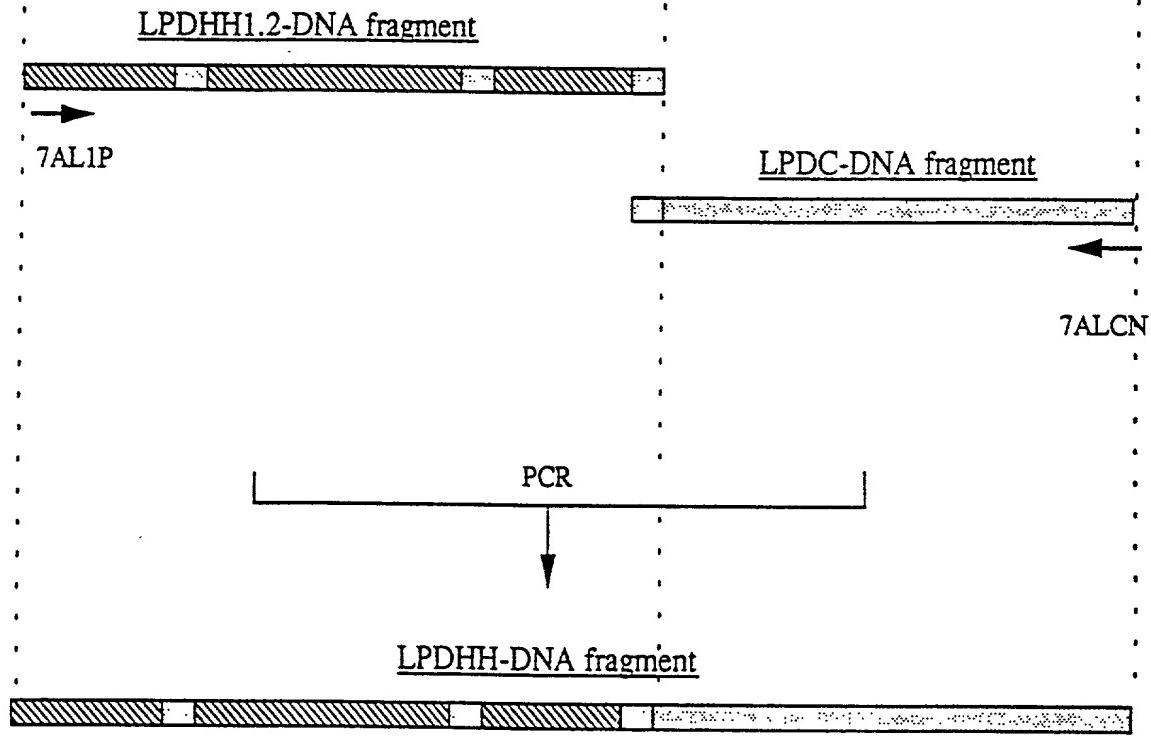


Fig. 33

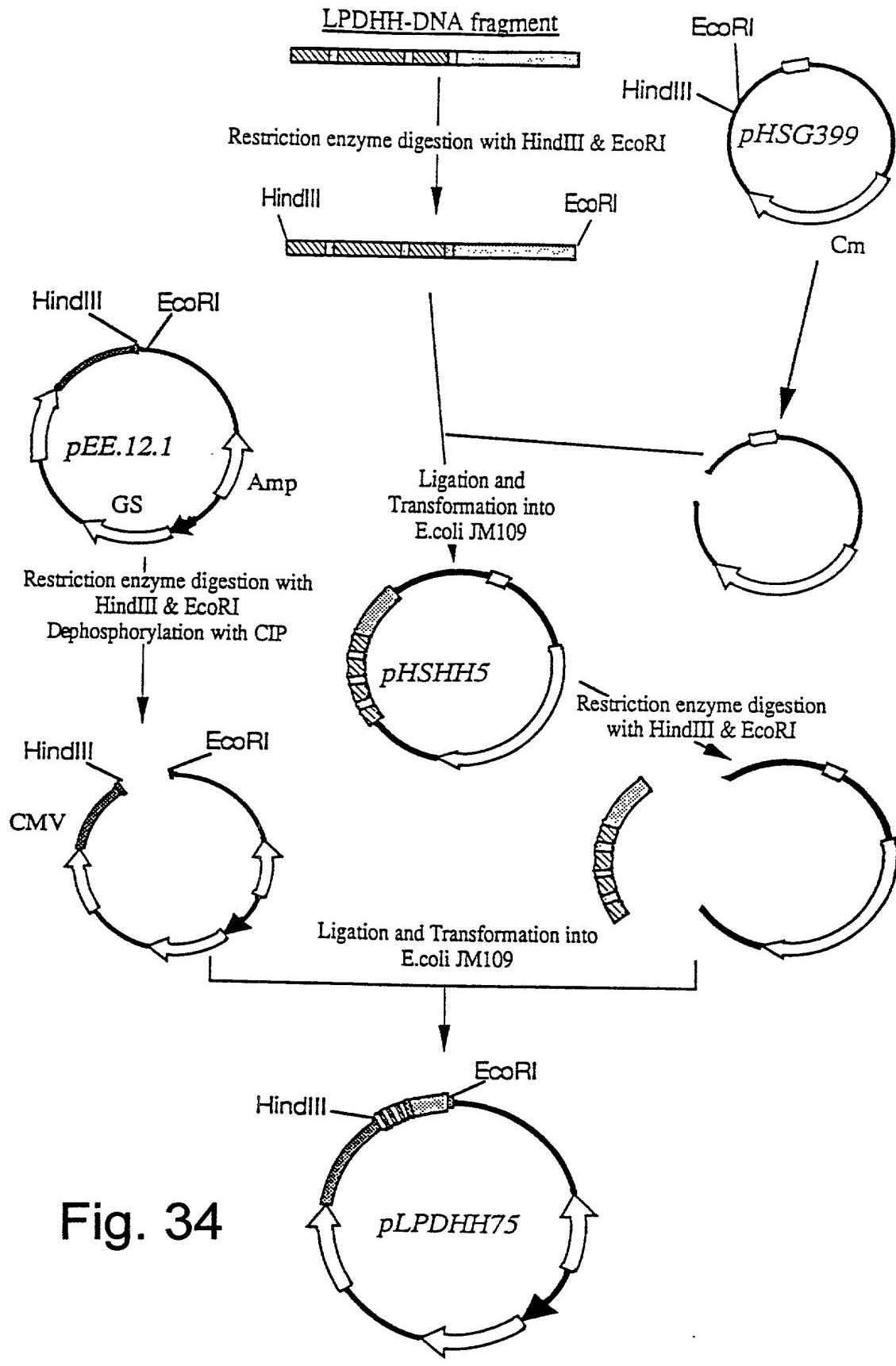


Fig. 34

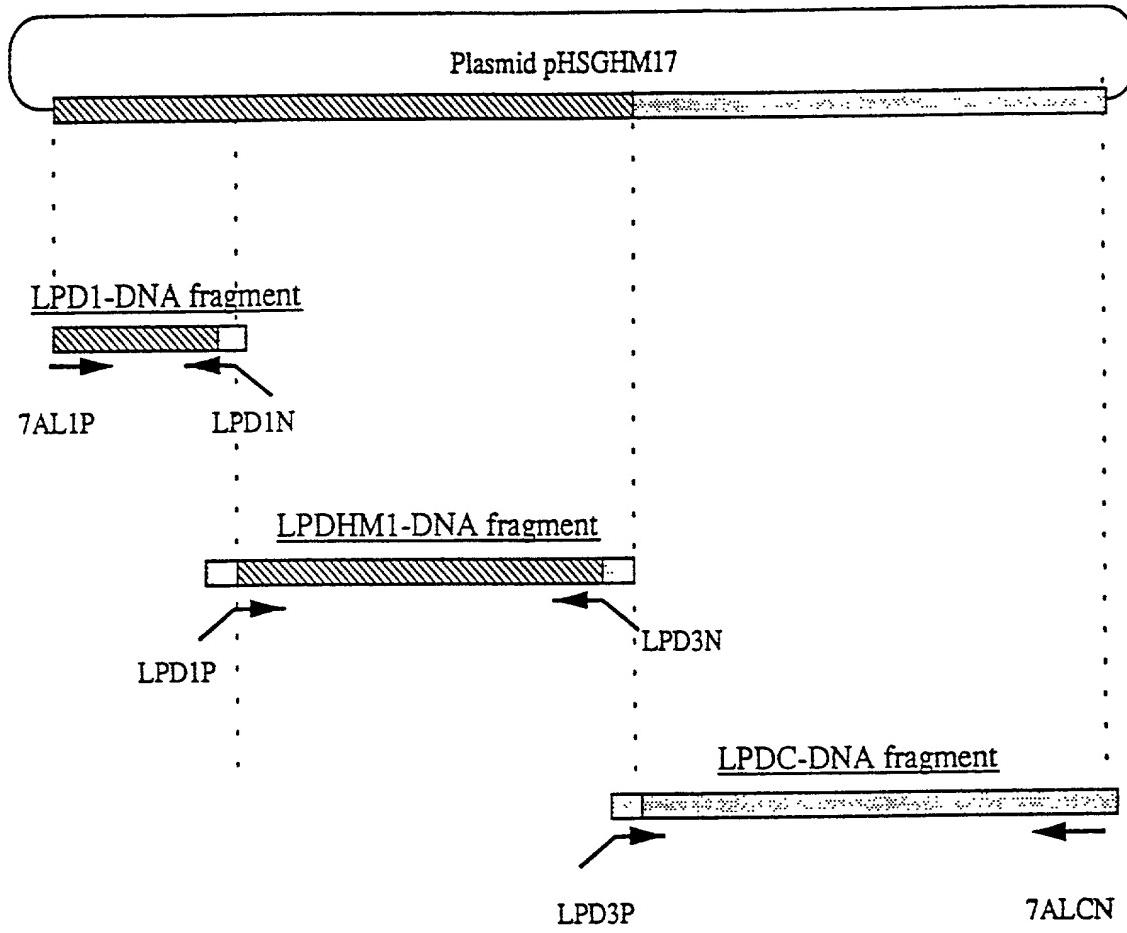
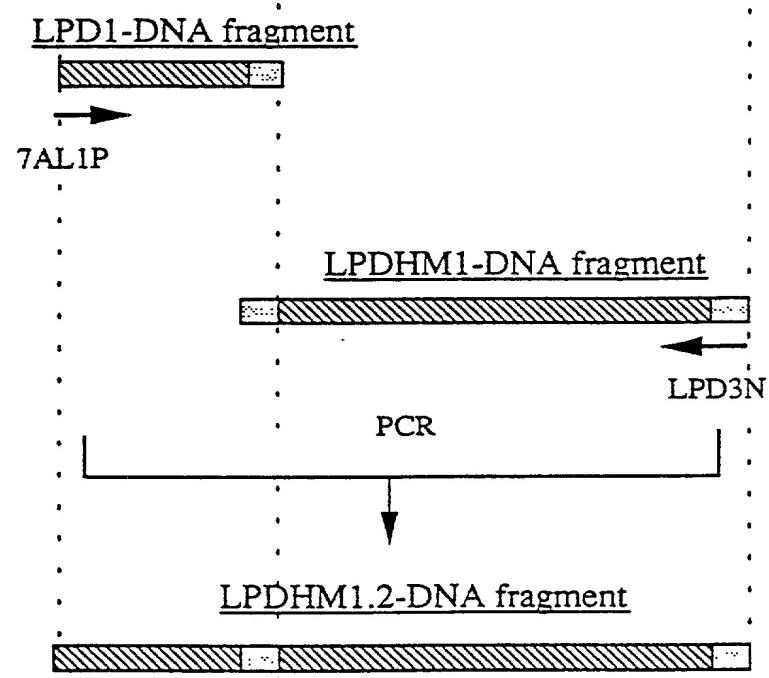


Fig. 35



**Fig. 36**

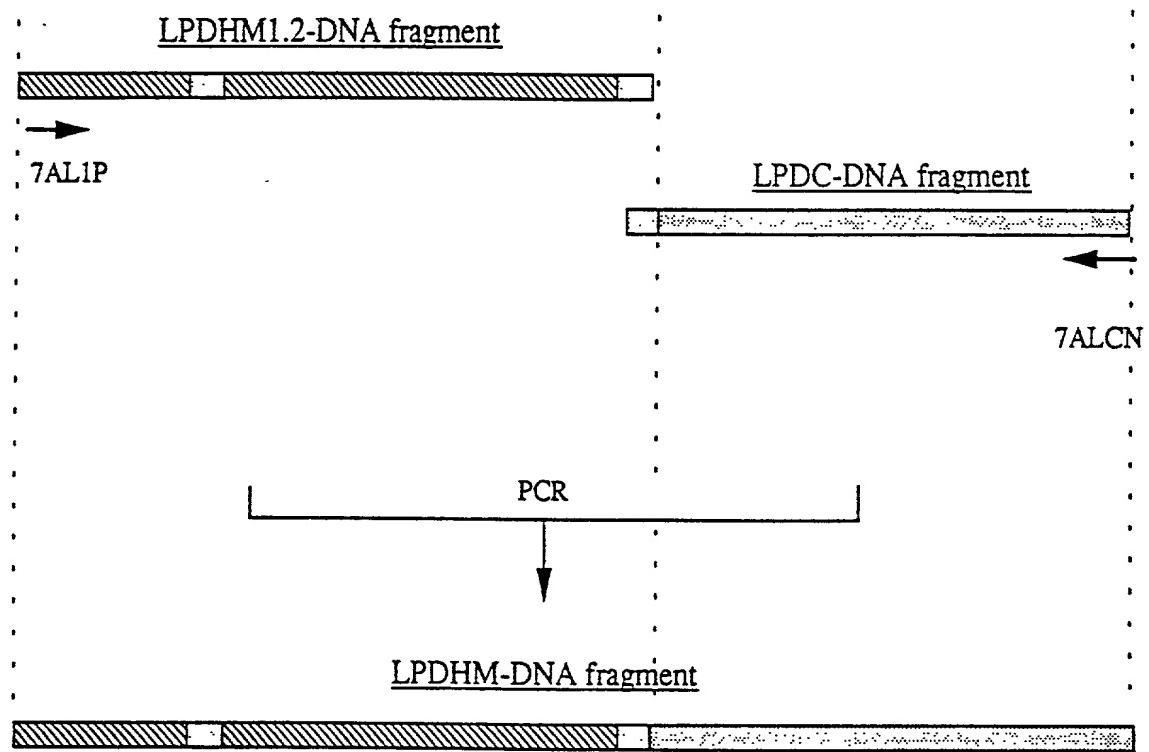
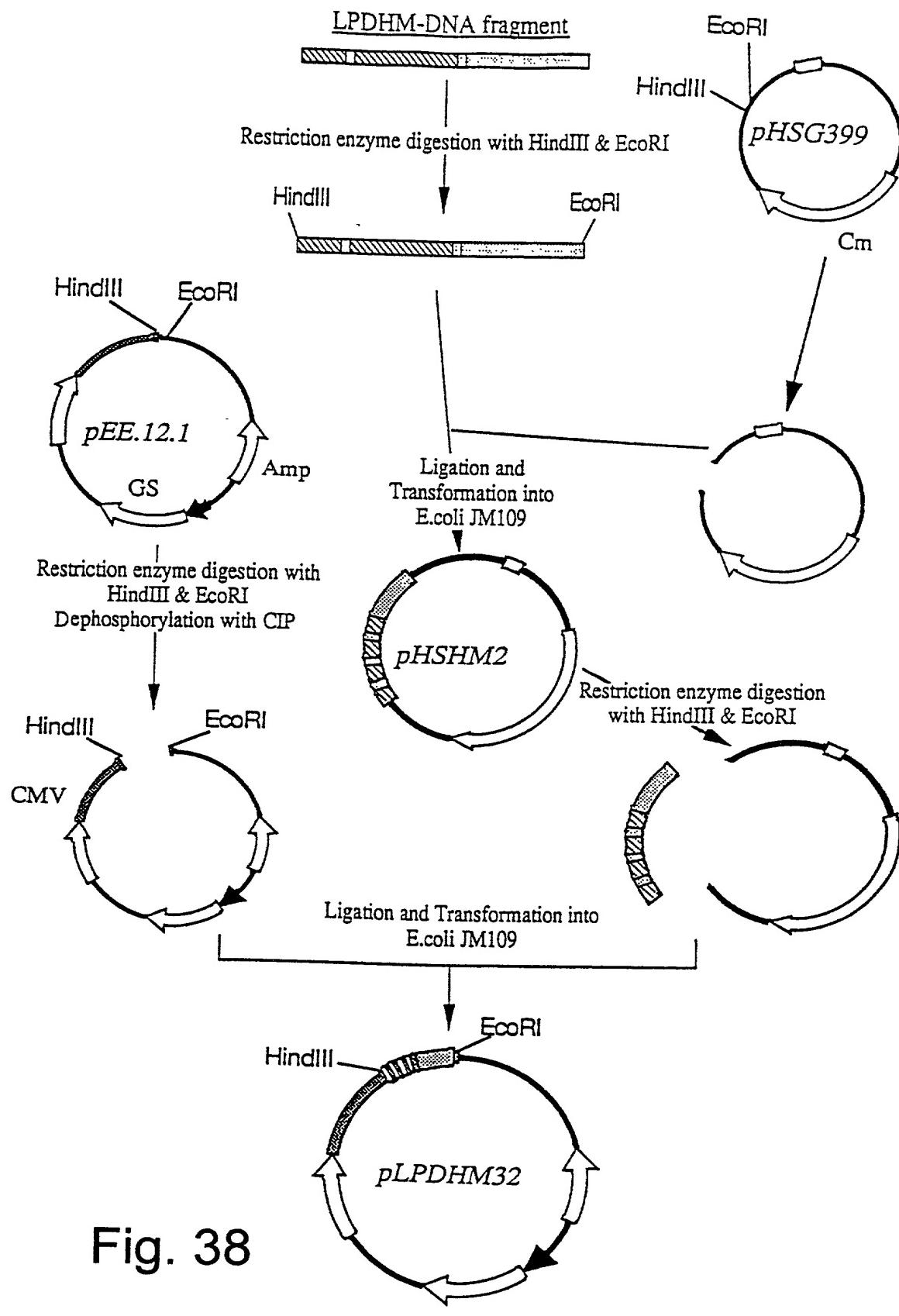


Fig. 37



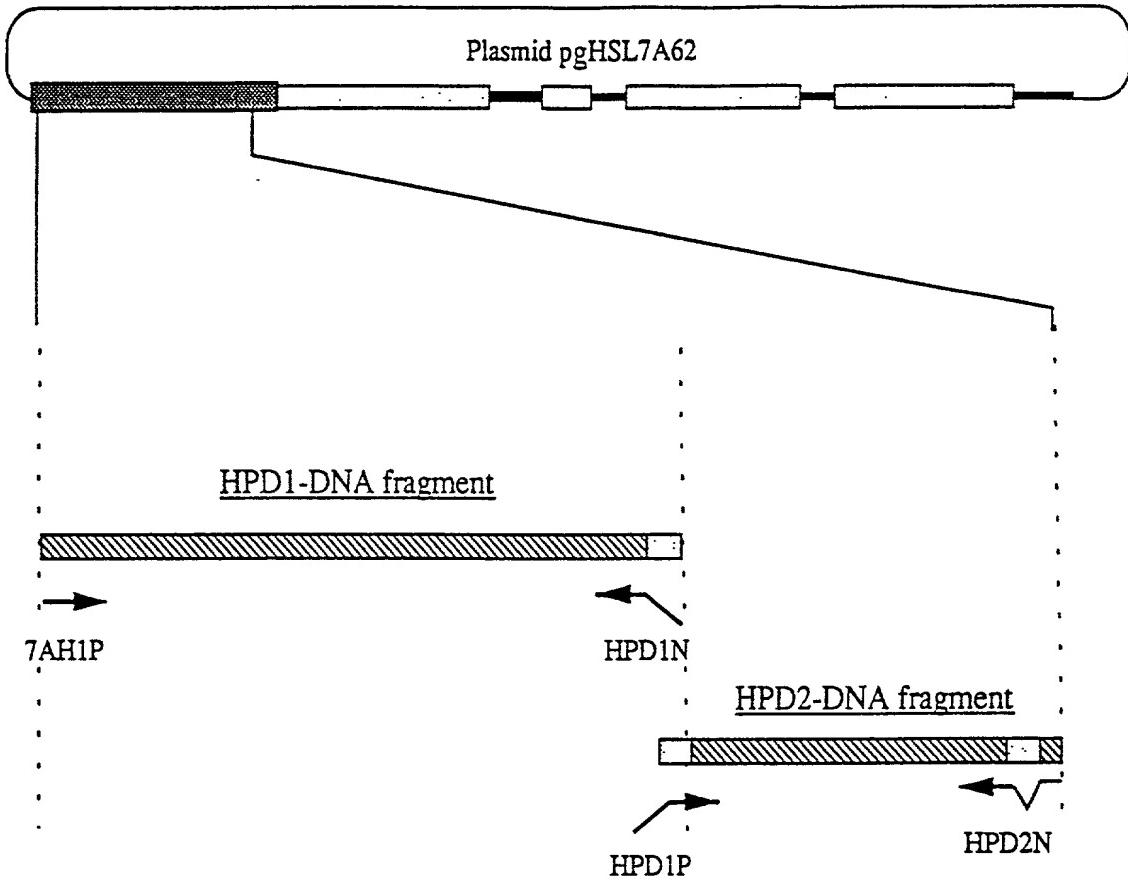
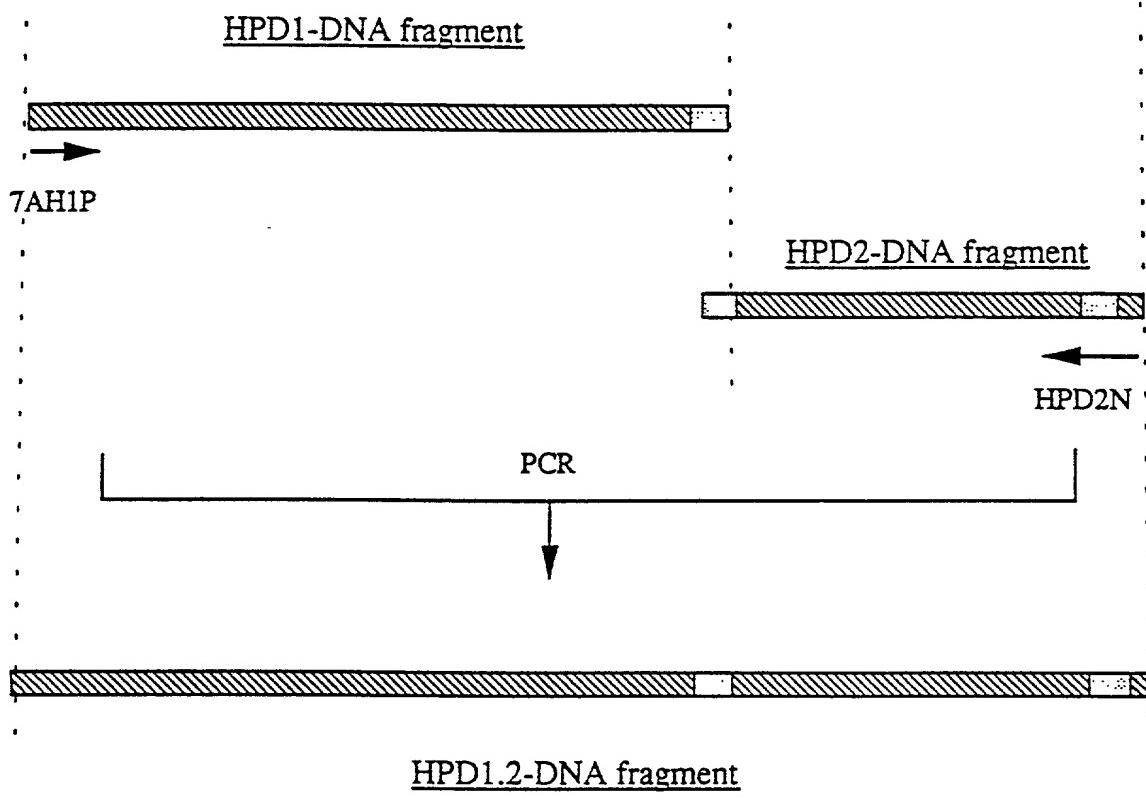


Fig. 39



**Fig. 40**

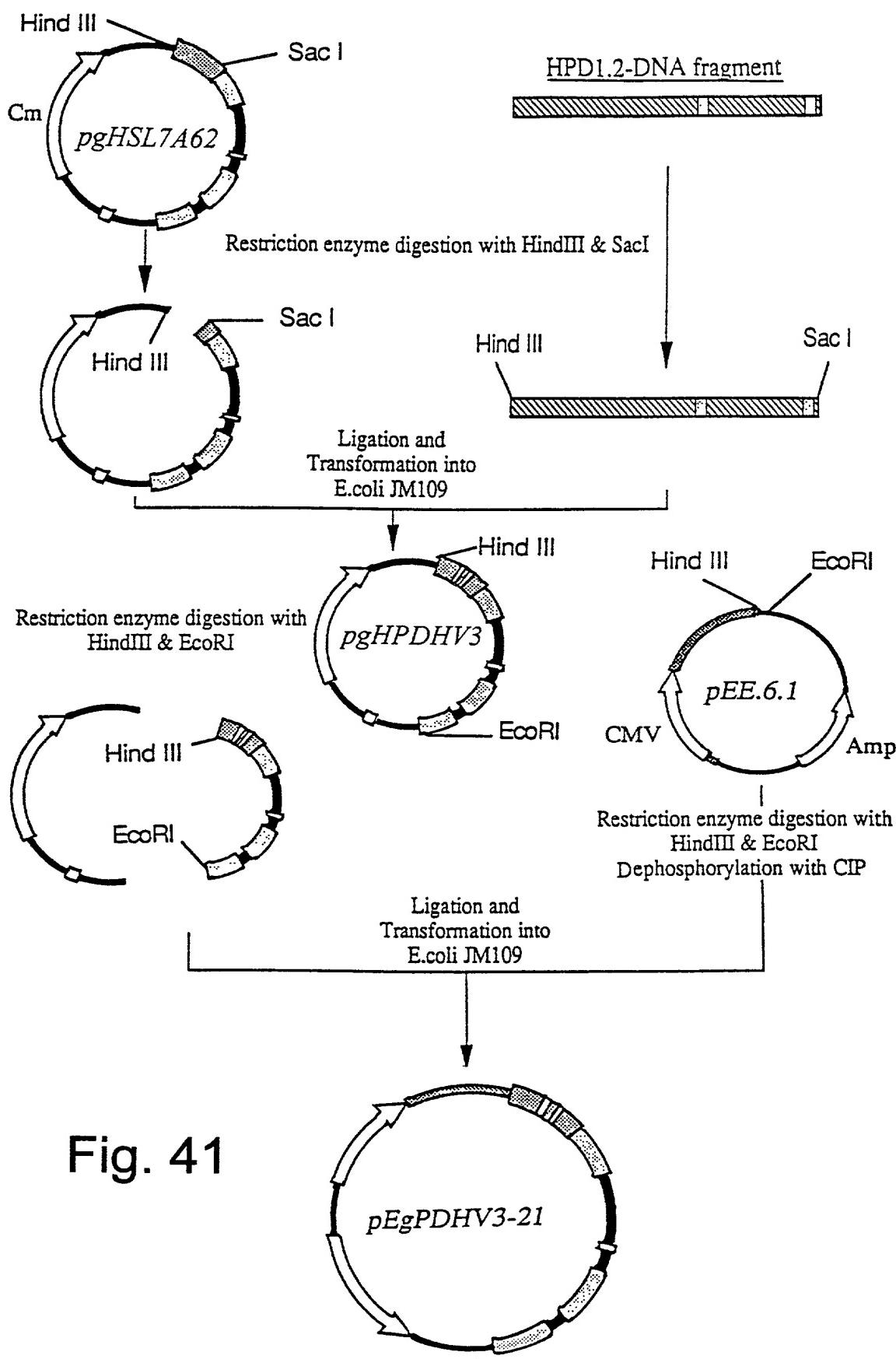


Fig. 41

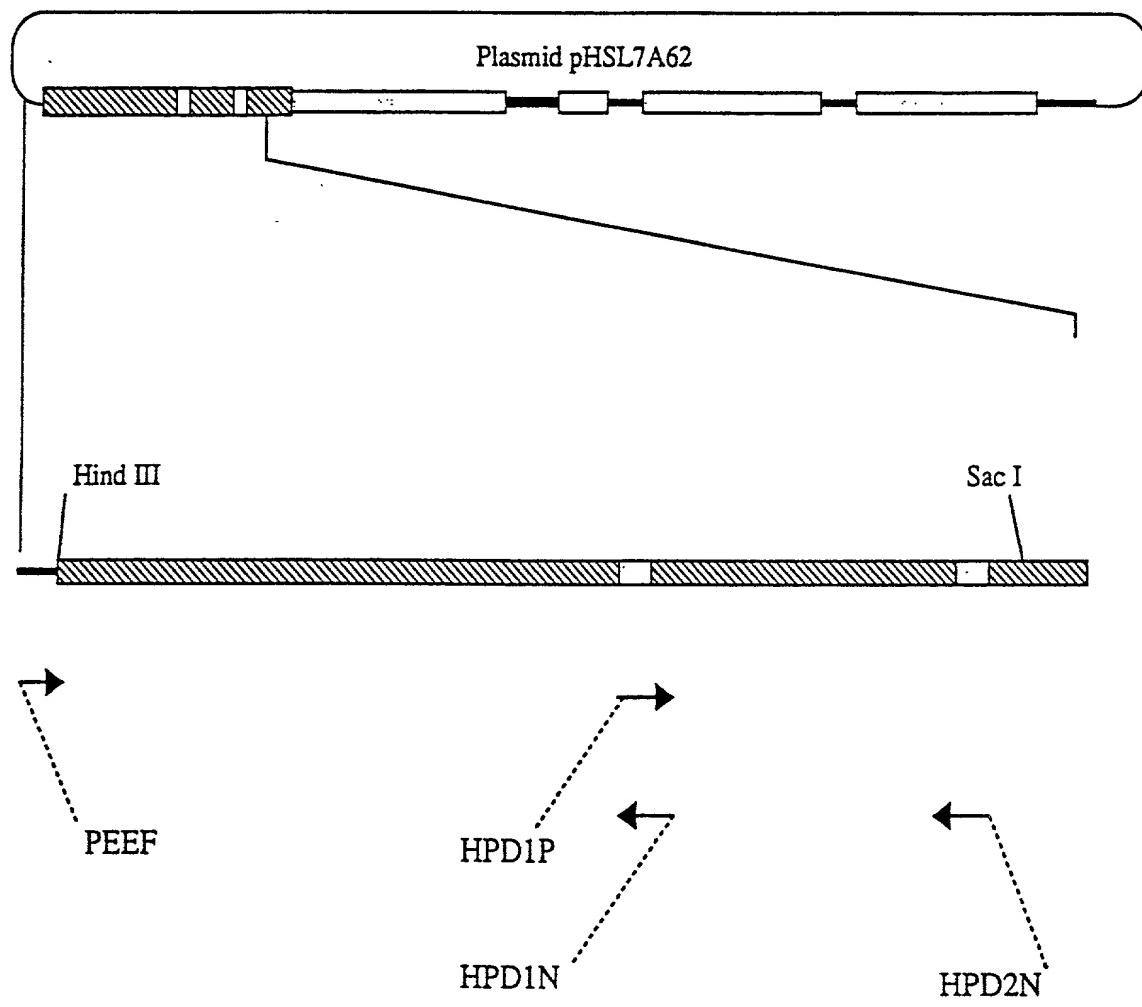


Fig. 42

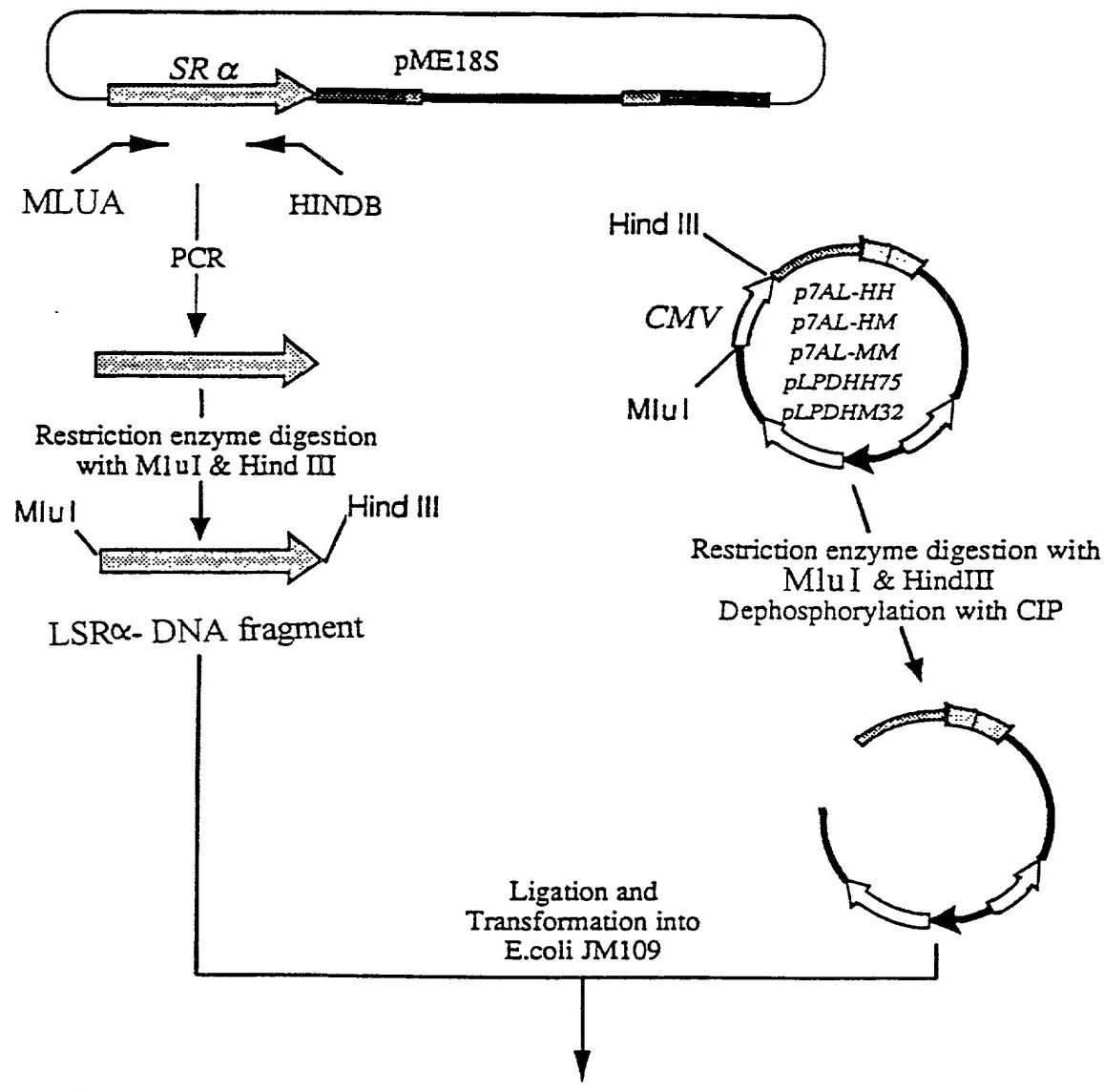
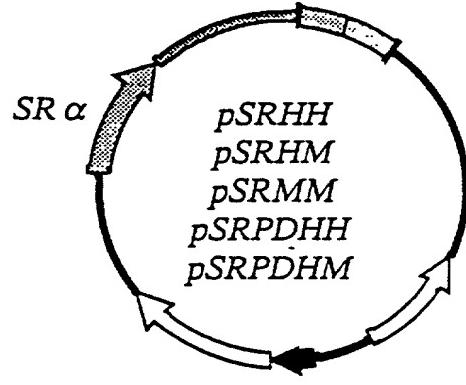


Fig. 43



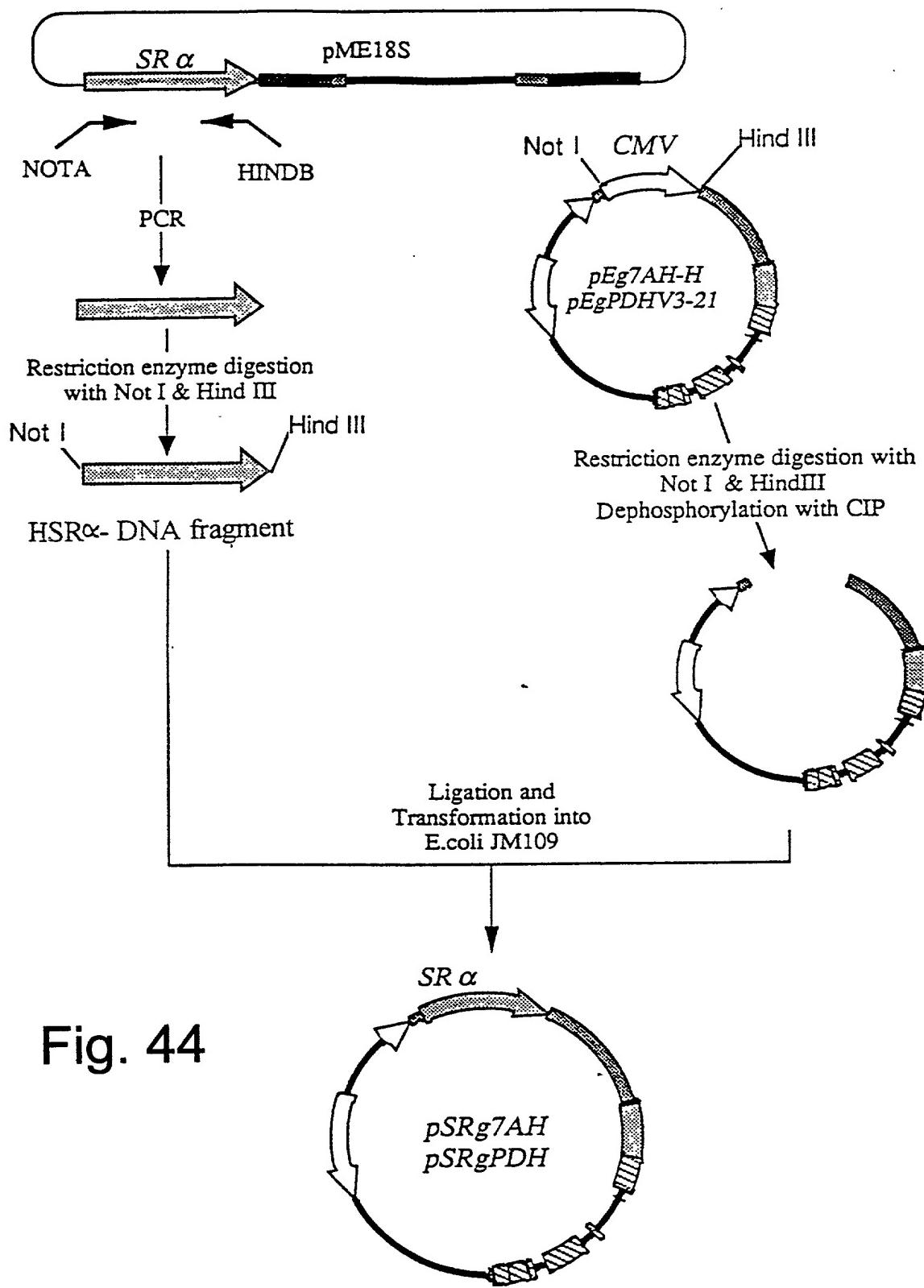


Fig. 44

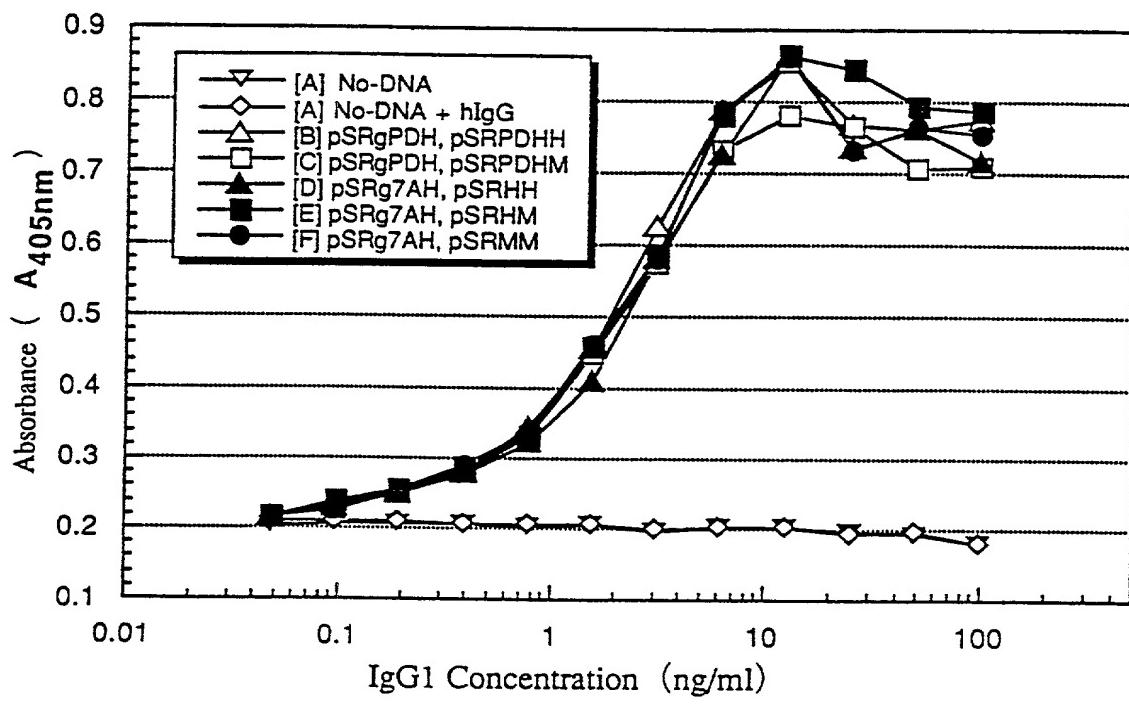


Fig. 45

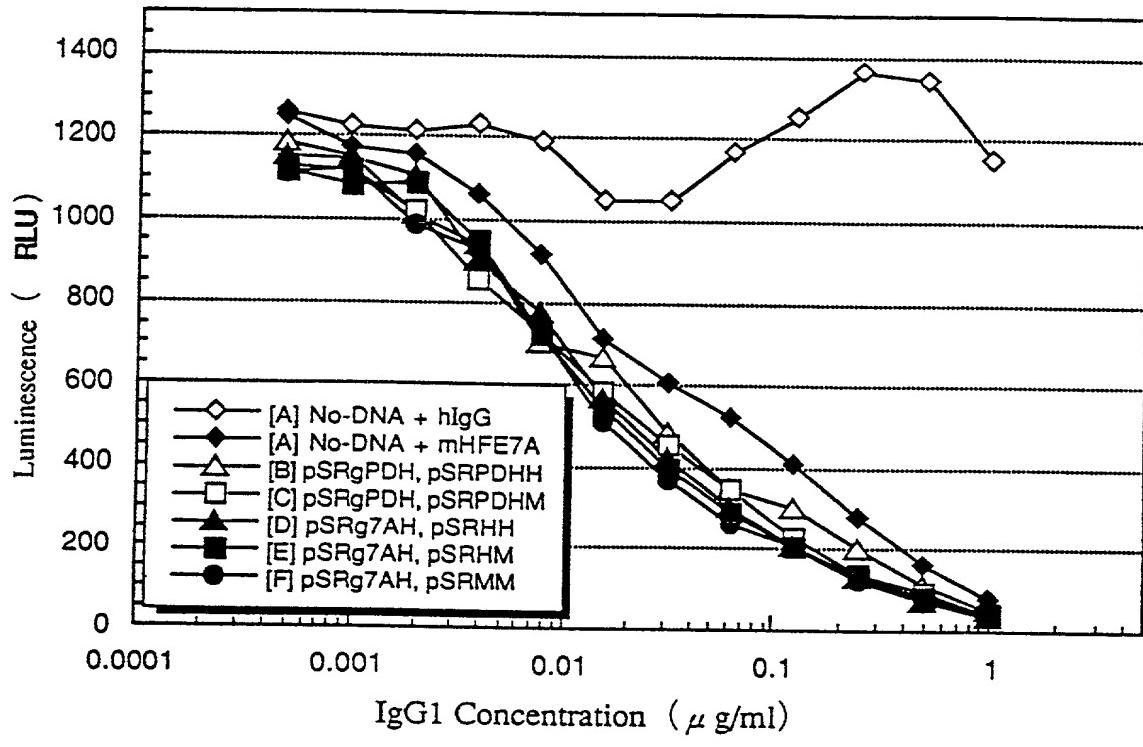


Fig. 46

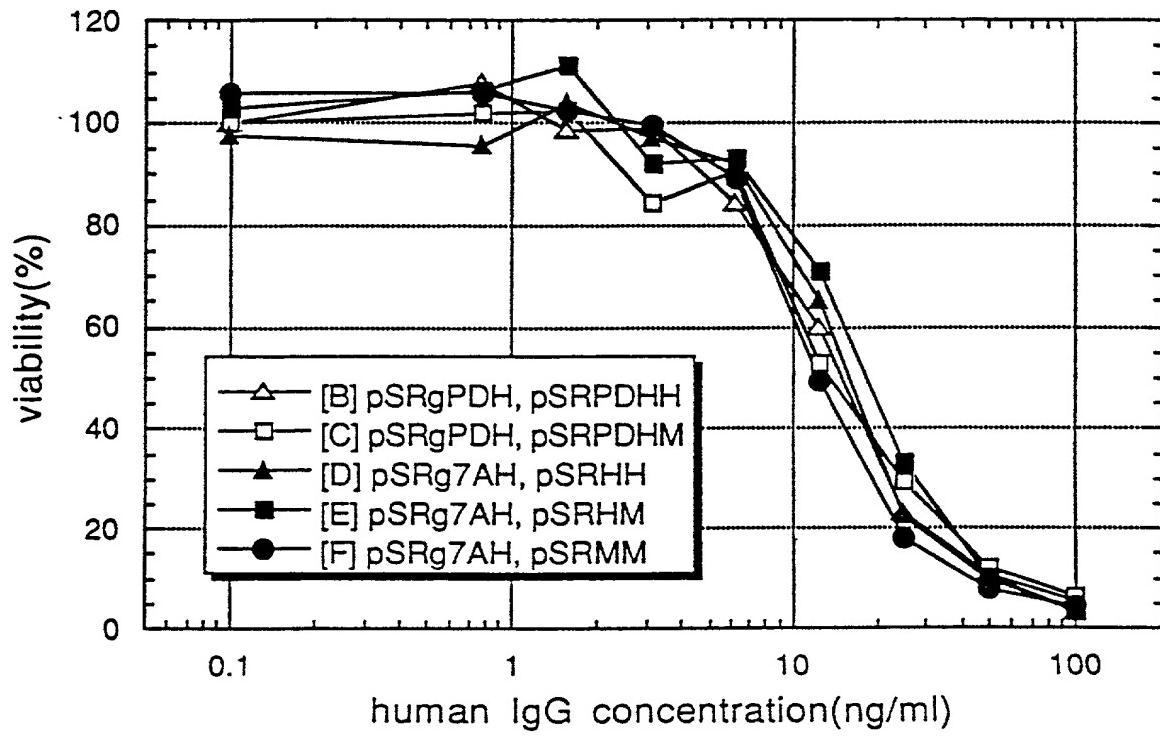


Fig. 47

# Fig. 48

<b>FRL<sub>1</sub></b>	1	5	10	15	20	
Mouse HFE7A	Asp	Ile	Val	Leu	Thr	Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys
Humanized HFE7A (8E10)	Asp	Ile	Val	Leu	Thr	Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu Arg Ala Thr Ile Ser Cys
Human Eu	Asp	Ile	Gin	Met	Thr	Gln Ser Pro Ser Thr Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
Humanized HFE7A (LEU1)	Asp	Ile	Val	Leu	Thr	Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
Humanized HFE7A (LEU2)	Asp	Ile	Val	Leu	Thr	Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys
Humanized HFE7A (LEU3)	Asp	Ile	Val	Leu	Thr	Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys

<b>FRL<sub>2</sub></b>	40	45	50			
Mouse HFE7A	Trp	Tyr	Gln	Lys	Pro	Gly
Humanized HFE7A (8E10)	Trp	Tyr	Gln	Gln	Pro	Gly
Human Eu	Trp	Tyr	Gln	Gln	Xaa	Pro
Humanized HFE7A (LEU1)	Trp	Tyr	Gln	Gln	Xaa	Pro
Humanized HFE7A (LEU2)	Trp	Tyr	Gln	Gln	Xaa	Pro
Humanized HFE7A (LEU3)	Trp	Tyr	Gln	Gln	Xaa	Pro

(Amino acid No. 47: Ala or Pro, No. 49: Lys or Arg)

<b>FRL<sub>3</sub></b>	65	70	75	80	85	90
Mouse HFE7A	Gly	Ile	Pro	Ala	Arg	Phe
Humanized HFE7A (8E10)	Gly	Ile	Pro	Asp	Arg	Thr
Human Eu	Gly	Val	Pro	Ser	Arg	Leu
Humanized HFE7A (LEU1)	Gly	Val	Pro	Ser	Ile	Gly
Humanized HFE7A (LEU2)	Gly	Val	Pro	Ser	Ile	Gly
Humanized HFE7A (LEU3)	Gly	Ile	Pro	Ser	Ile	Gly

<b>FRL<sub>4</sub></b>	105	110				
Mouse HFE7A	Phe	Gly	Gly	Thr	Lys	Leu
Humanized HFE7A (8E10)	Phe	Gly	Gly	Arg	Leu	Glu
Human Eu	Phe	Gly	Gln	Gly	Thr	Lys
Humanized HFE7A (LEU1)	Phe	Gly	Gln	Gly	Thr	Val
Humanized HFE7A (LEU2)	Phe	Gly	Gln	Gly	Thr	Val
Humanized HFE7A (LEU3)	Phe	Gly	Gln	Gly	Thr	Val

**Fig. 49**

<b>FRH<sub>1</sub></b>		<b>FRH<sub>2</sub></b>		<b>FRH<sub>3</sub></b>		<b>FRH<sub>4</sub></b>	
1	5	10	15	20	25	30	35
Mouse HFE7A	Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr						
Humanized HFE7A(8E10)	Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr						
Human Eu	Glu Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Pro Gly Ser Ser Val Lys Val Ser Cys Lys Ala Ser Gly Gly Thr Phe Ser						
Humanized HFE7A(HEU1)	Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr						
Humanized HFE7A(HEU2)	Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr						
Humanized HFE7A(HEU3)	Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr						
Mouse HFE7A	Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile Gly	40	45				
Humanized HFE7A(8E10)	Trp Val Lys Gln Ala Pro Gly Gln Arg Leu Glu Trp Met Gly						
Human Eu	Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly						
Humanized HFE7A(HEU1)	Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly						
Humanized HFE7A(HEU2)	Trp Val Lys Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly						
Humanized HFE7A(HEU3)	Trp Val Arg Gln Ala Pro Gly Gln Gly Leu Glu Trp Met Gly						
Mouse HFE7A	Lys Ala Thr Val Asp Thr Ser Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys Ala Arg	70	75	80	85	90	95
Humanized HFE7A(8E10)	Lys Ala Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg						
Human Eu	Arg Val Thr Ile Thr Ala Asp Glu Ser Thr Asn Thr Ala Tyr Met Gln Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Phe Tyr Phe Cys Ala Gly						
Humanized HFE7A(HEU1)	Lys Ala Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg						
Humanized HFE7A(HEU2)	Lys Ala Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg						
Humanized HFE7A(HEU3)	Lys Ala Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr Met Gln Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg						

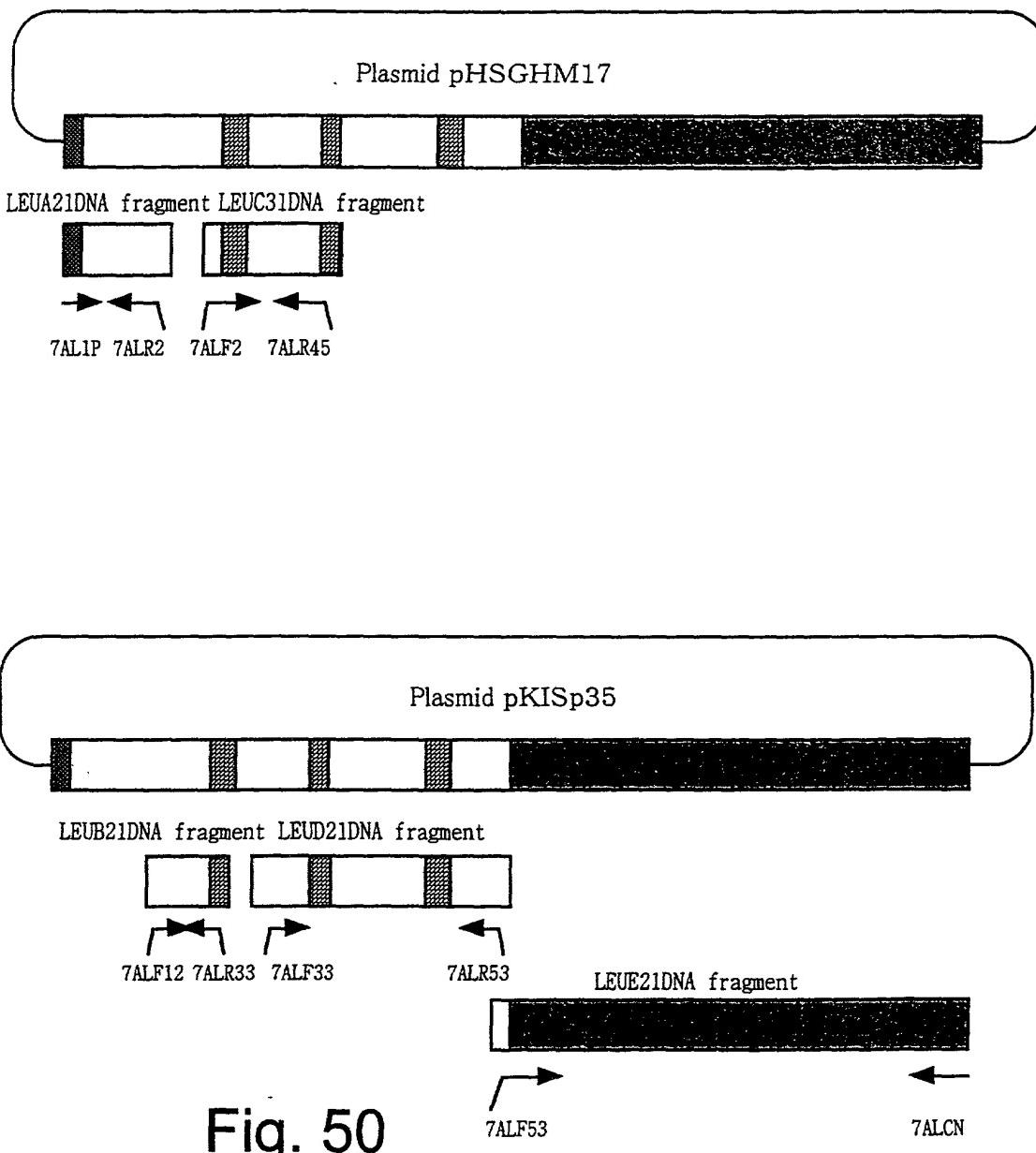
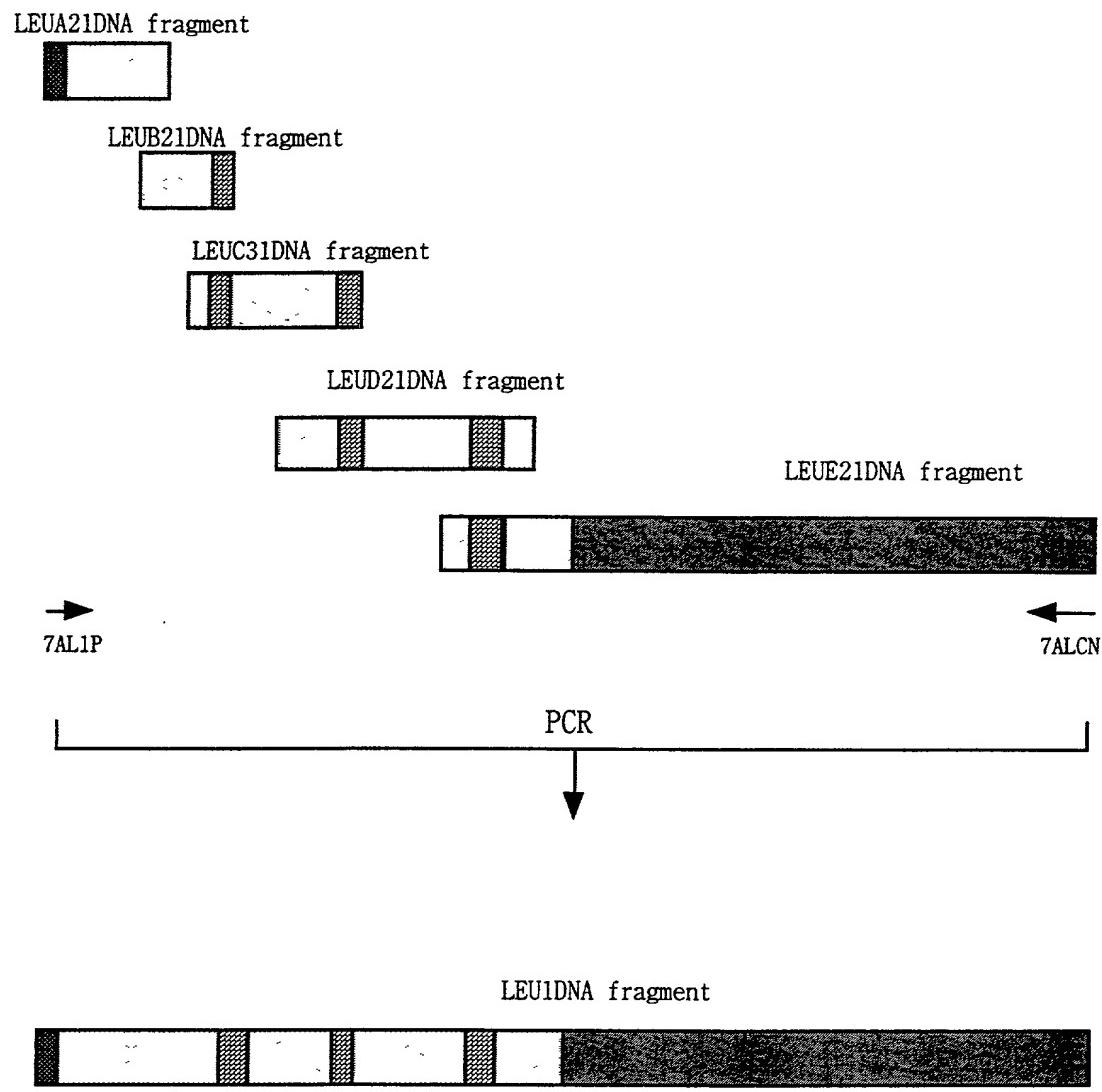
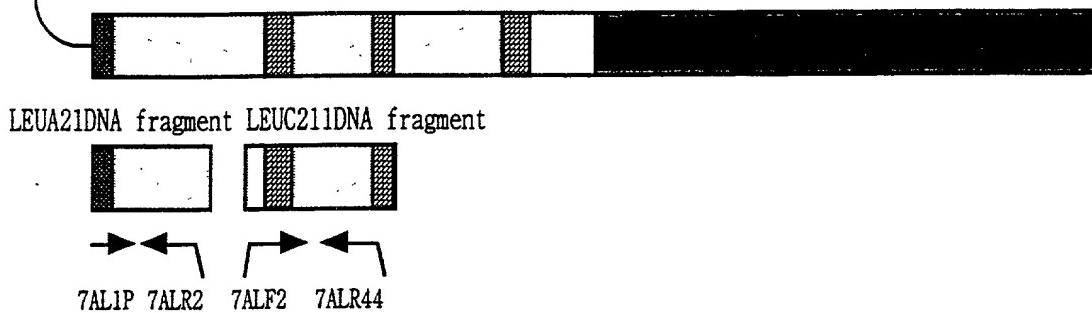


Fig. 50



**Fig. 51**

Plasmid pHSGHM17



Plasmid pKISp35

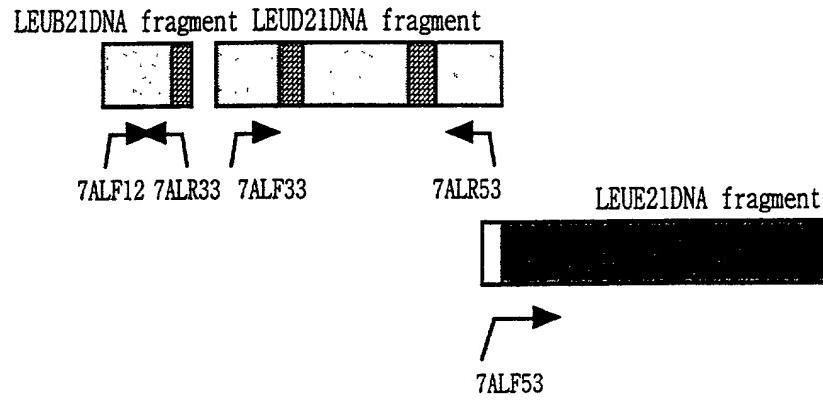
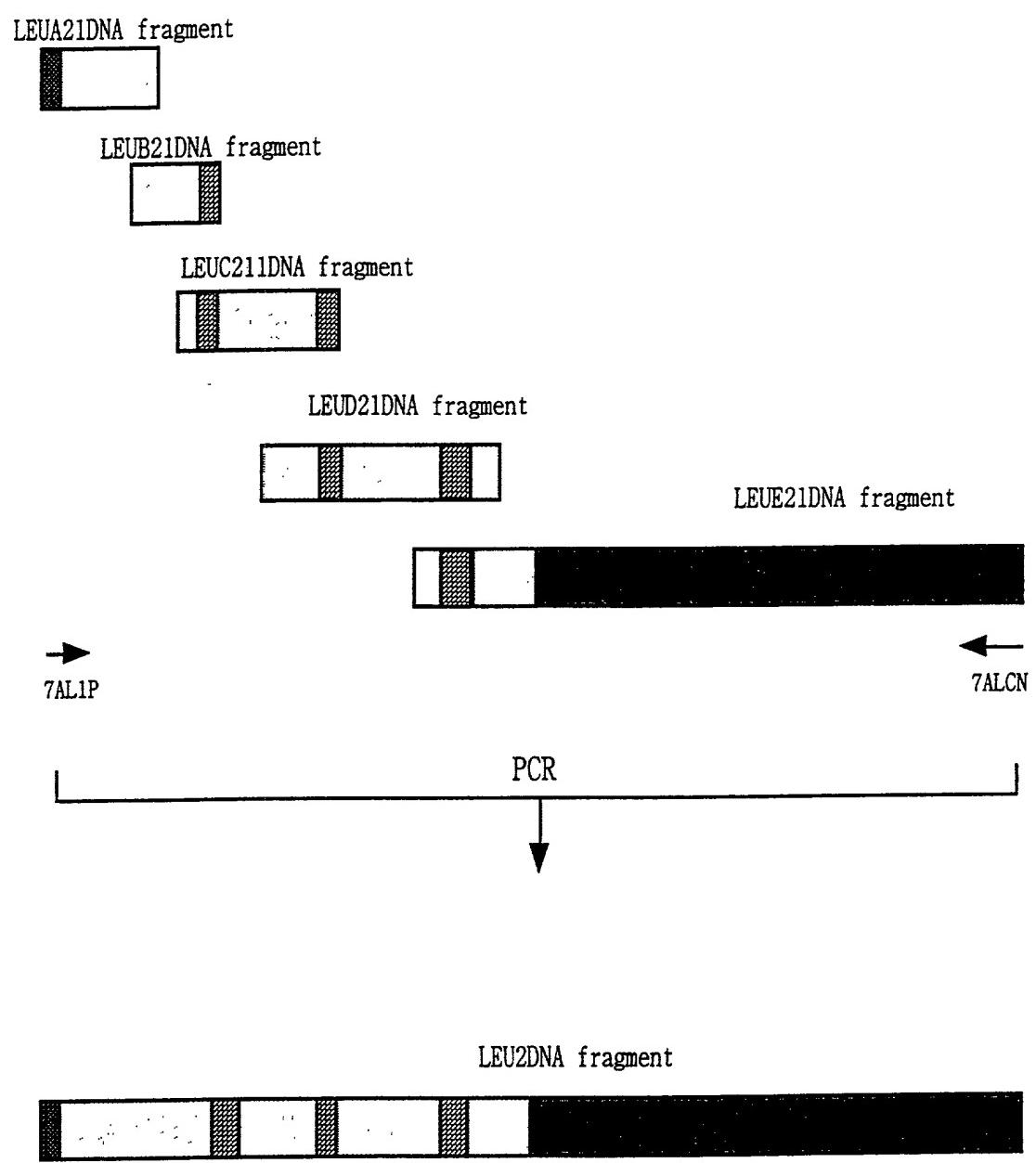
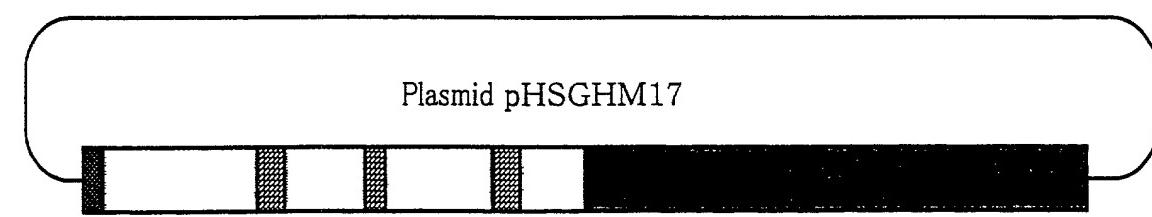


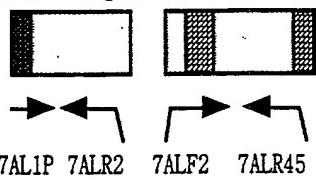
Fig. 52



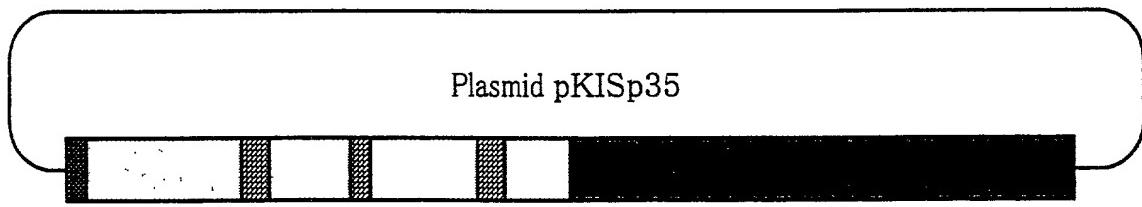
**Fig. 53**



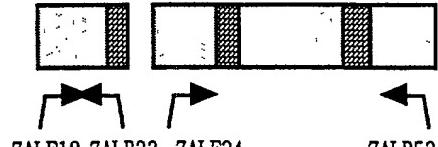
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7AL1P 7ALR2    7ALF2 7ALR45



LEUB21DNA fragment LEUD31DNA fragment



7ALF12 7ALR33 7ALF34

7ALR53

LEUE21DNA fragment



7ALF53

7ALCN

**Fig. 54**

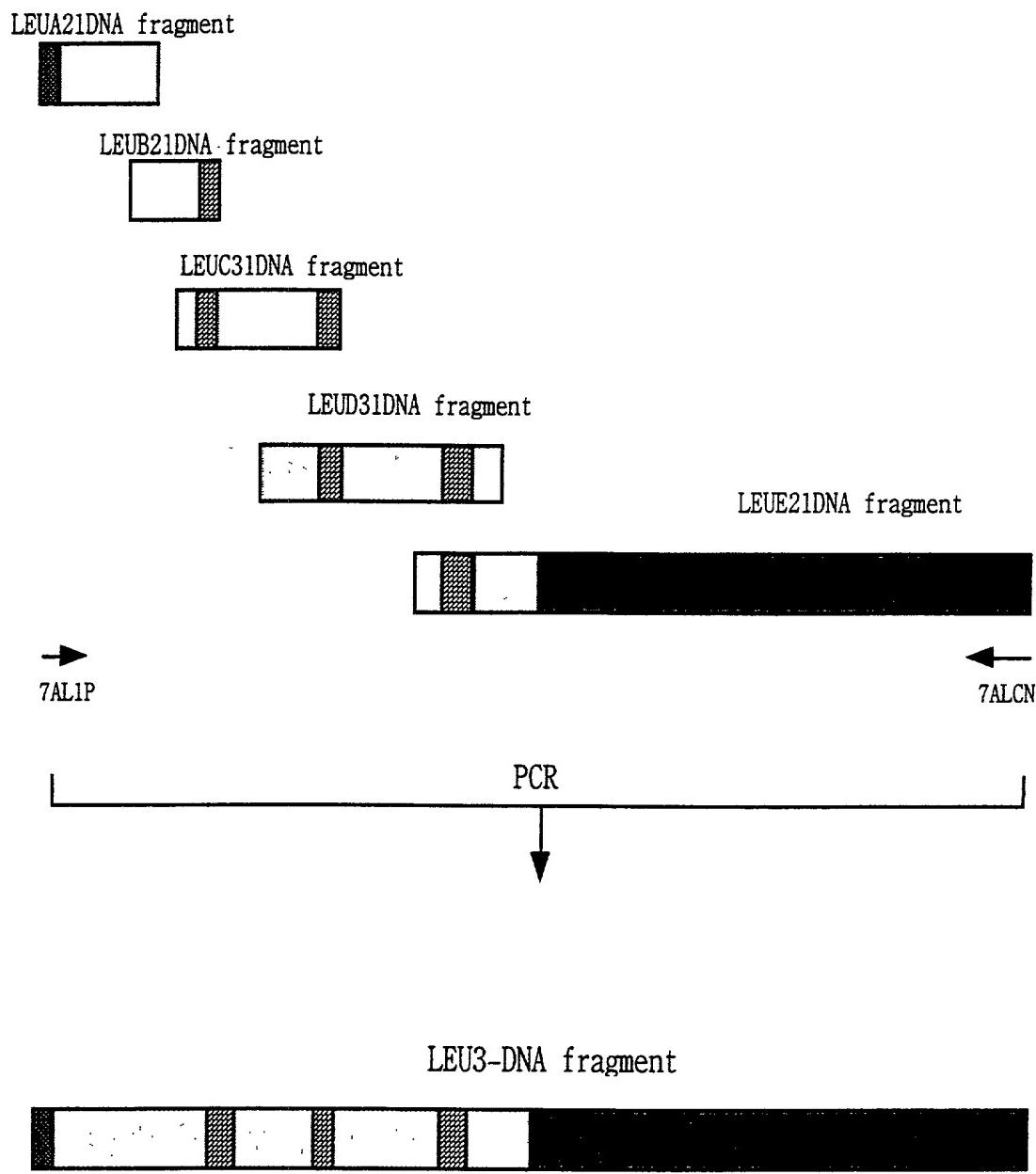


Fig. 55

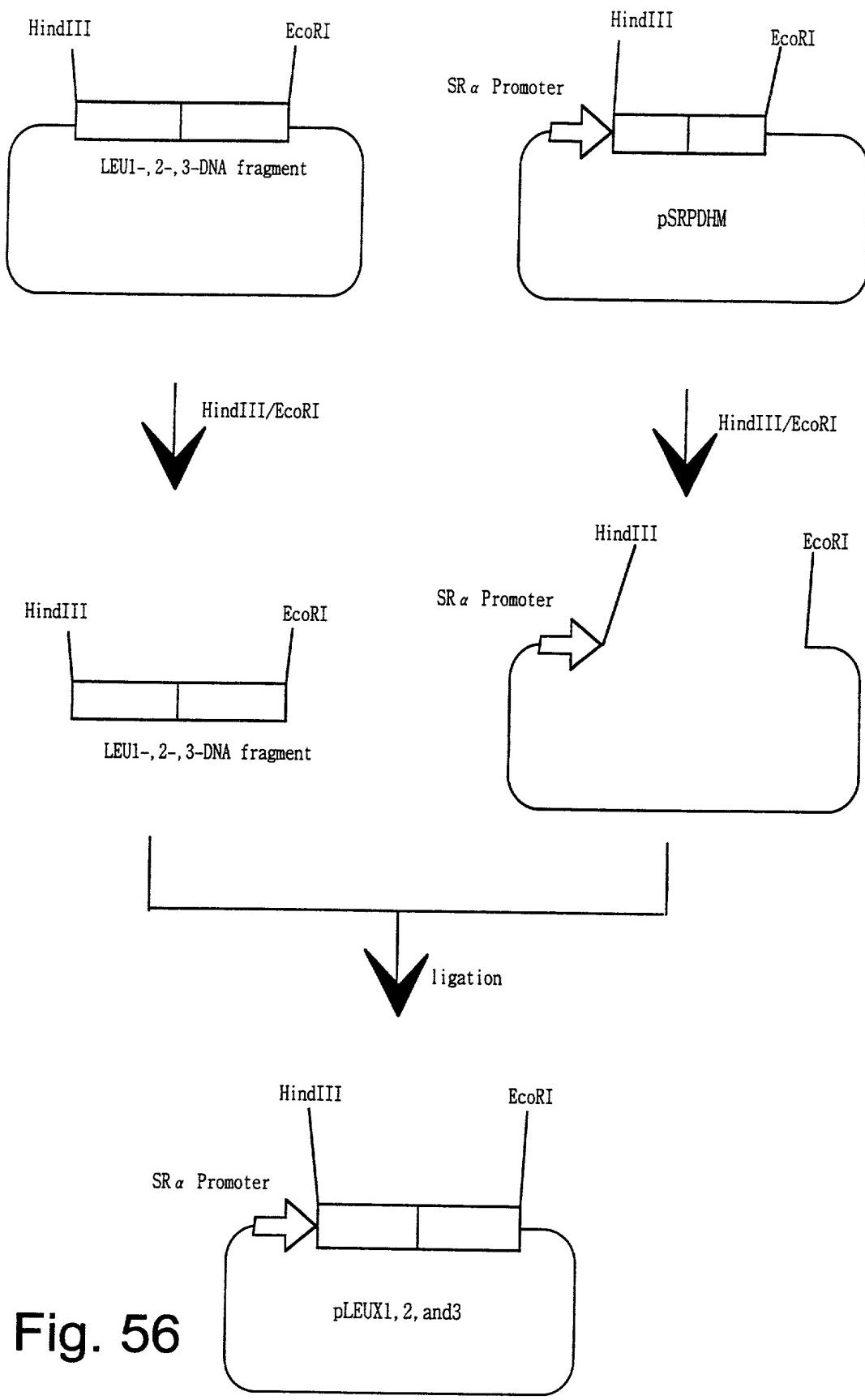
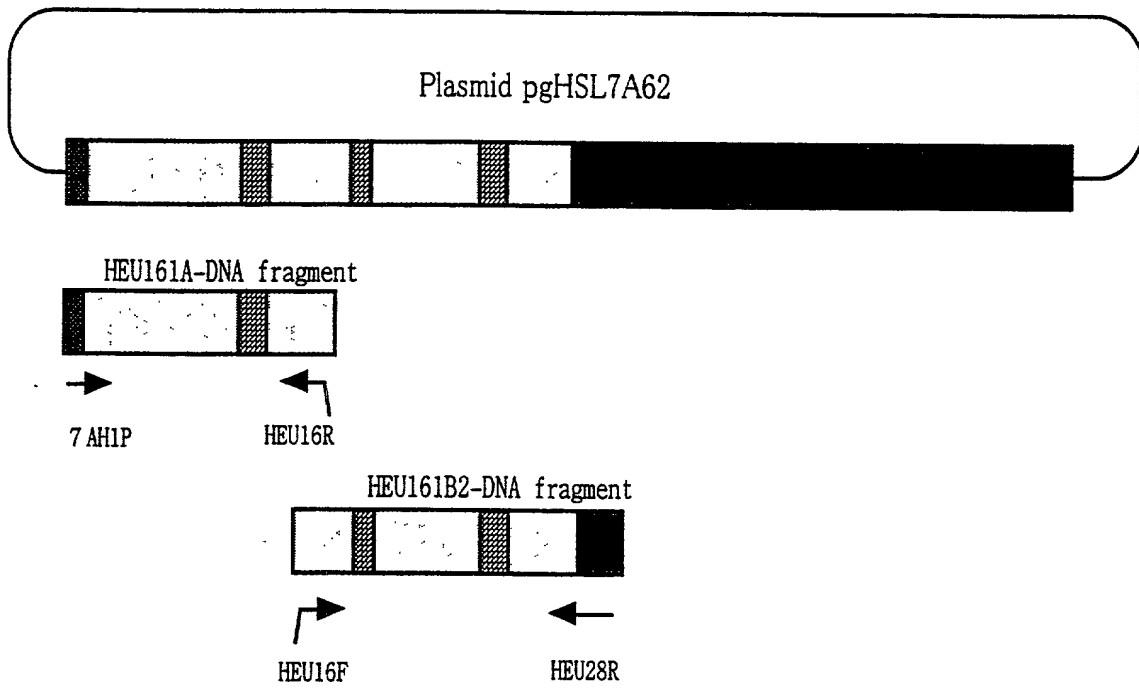
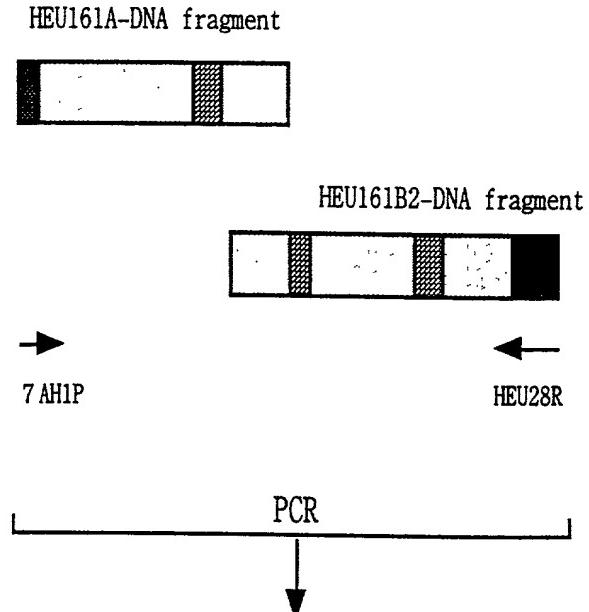


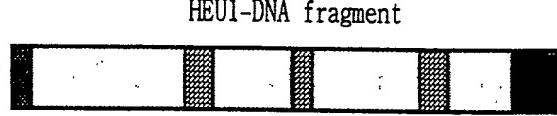
Fig. 56



**Fig. 57**



**Fig. 58**



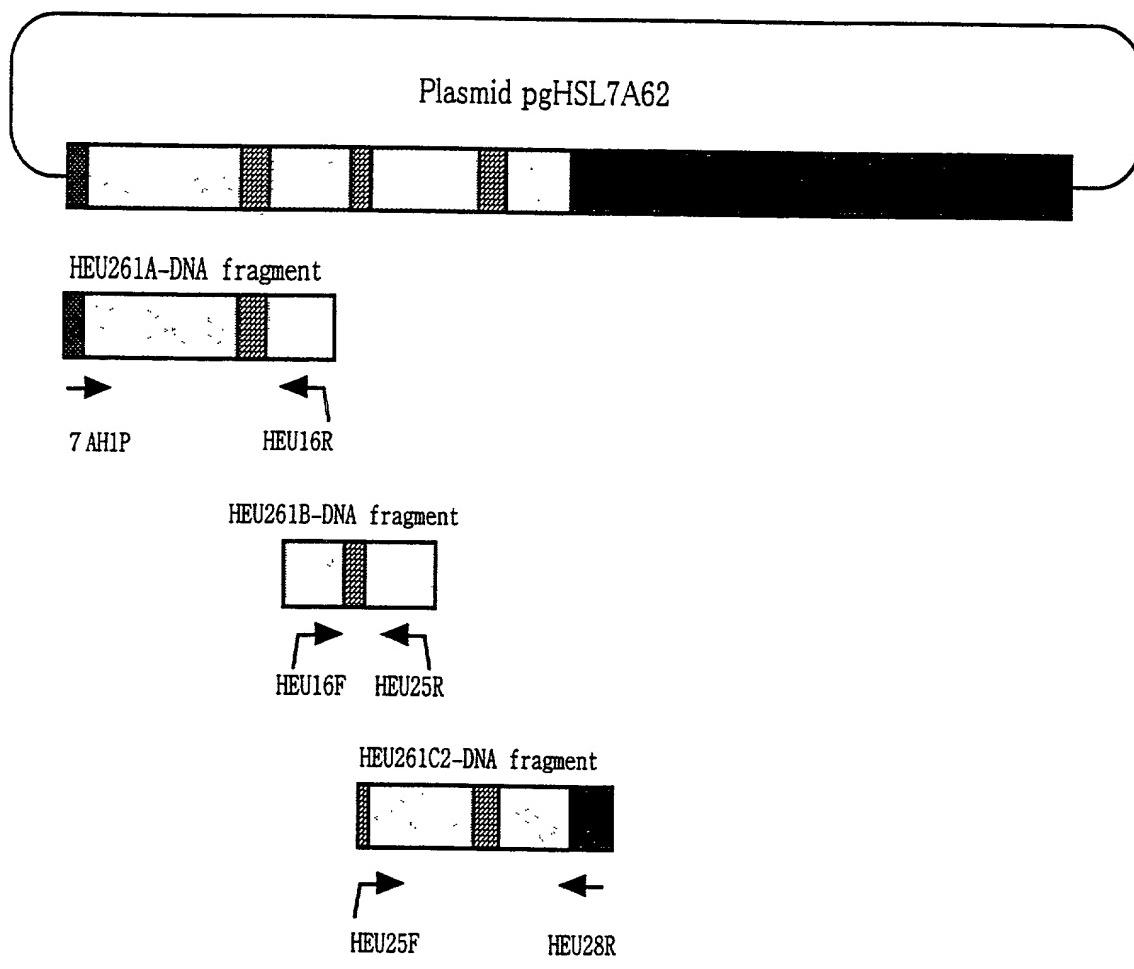


Fig. 59

Plasmid pgHSL7A62



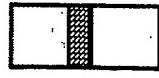
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7 AH1P

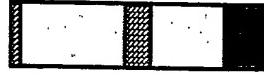
HEU16R

HEU261B-DNA fragment



HEU16F      HEU25R

HEU261C2-DNA fragment



HEU25F

HEU28R

Fig. 60

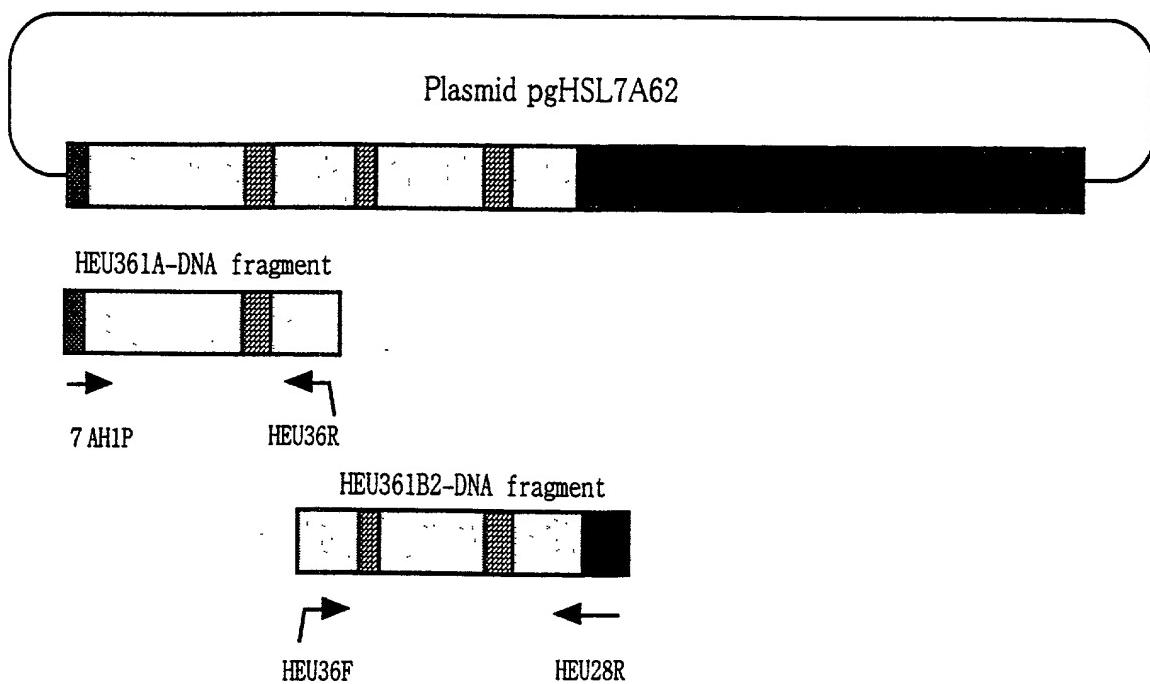


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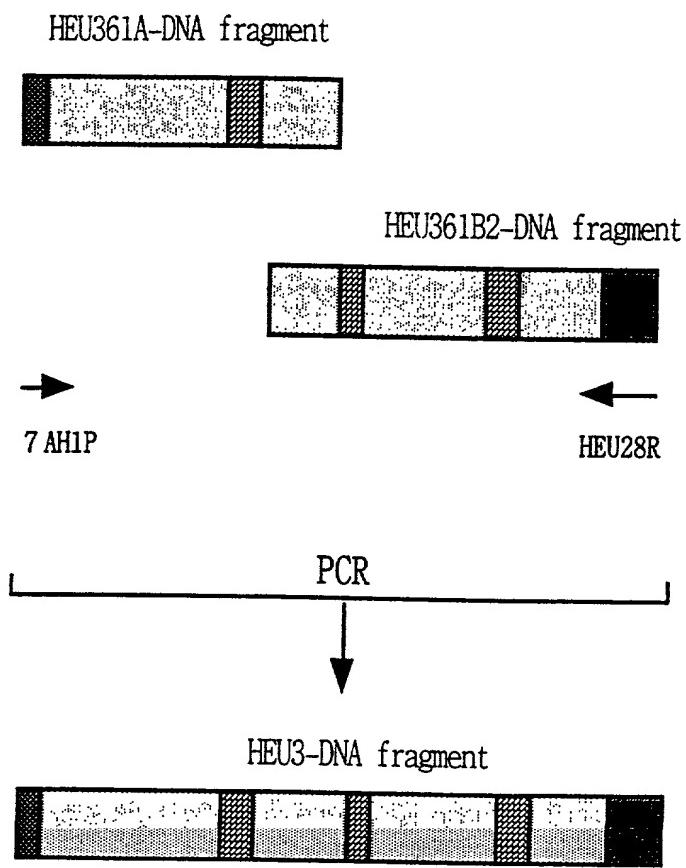


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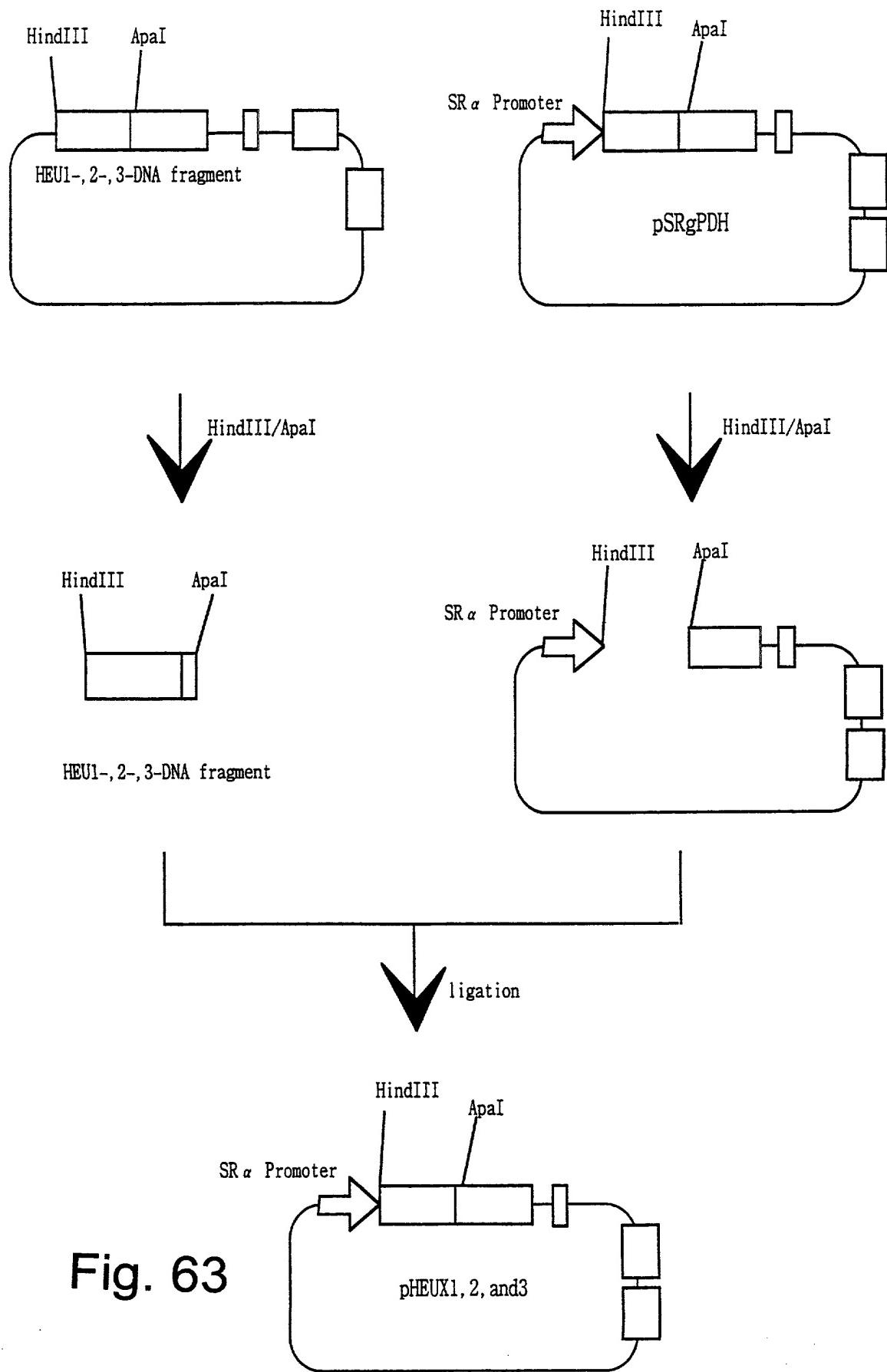


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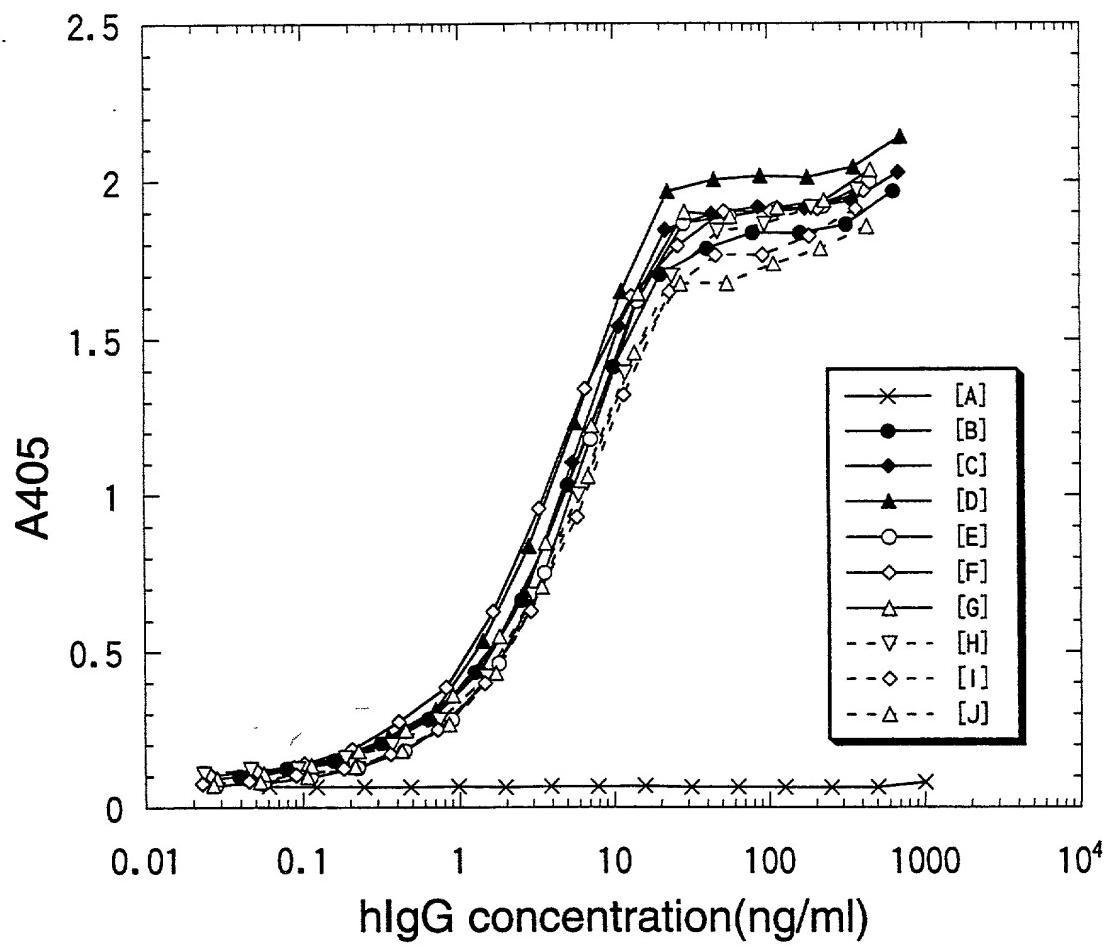


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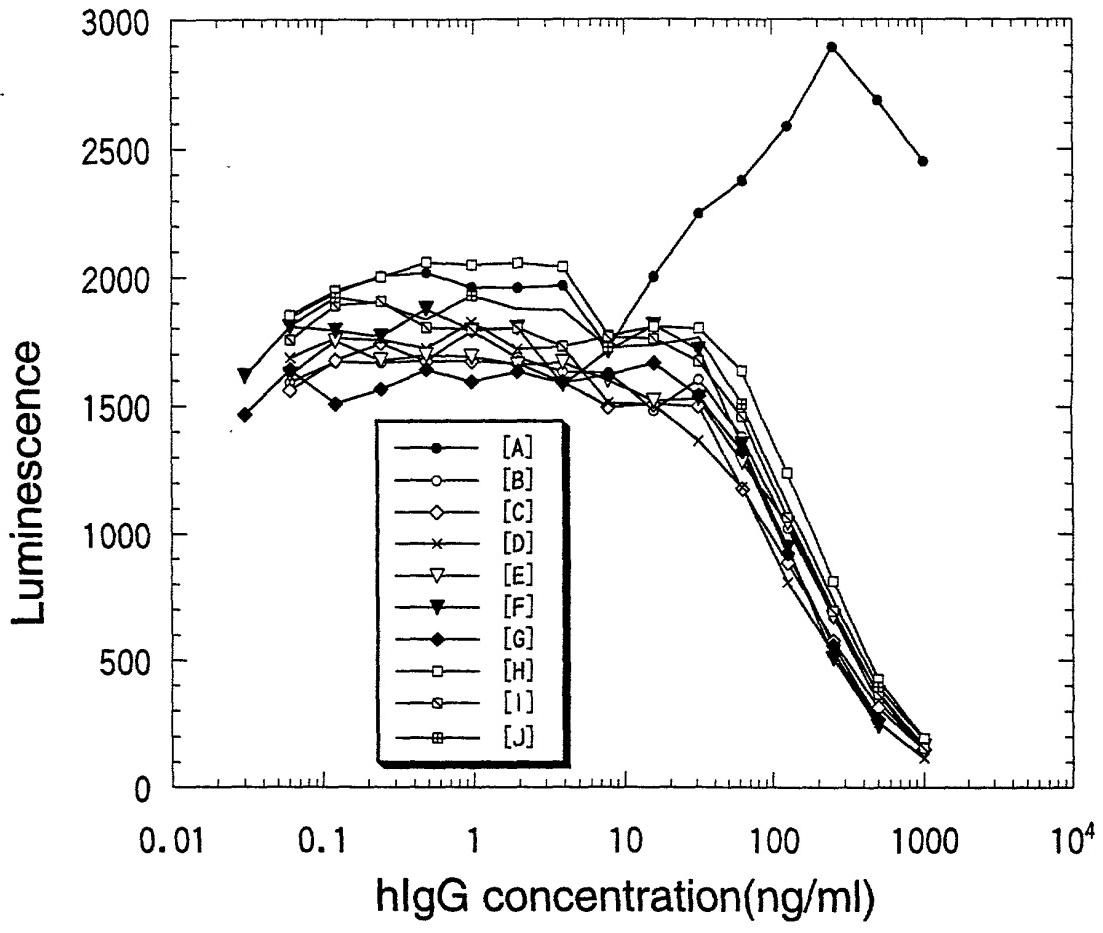


Fig. 65

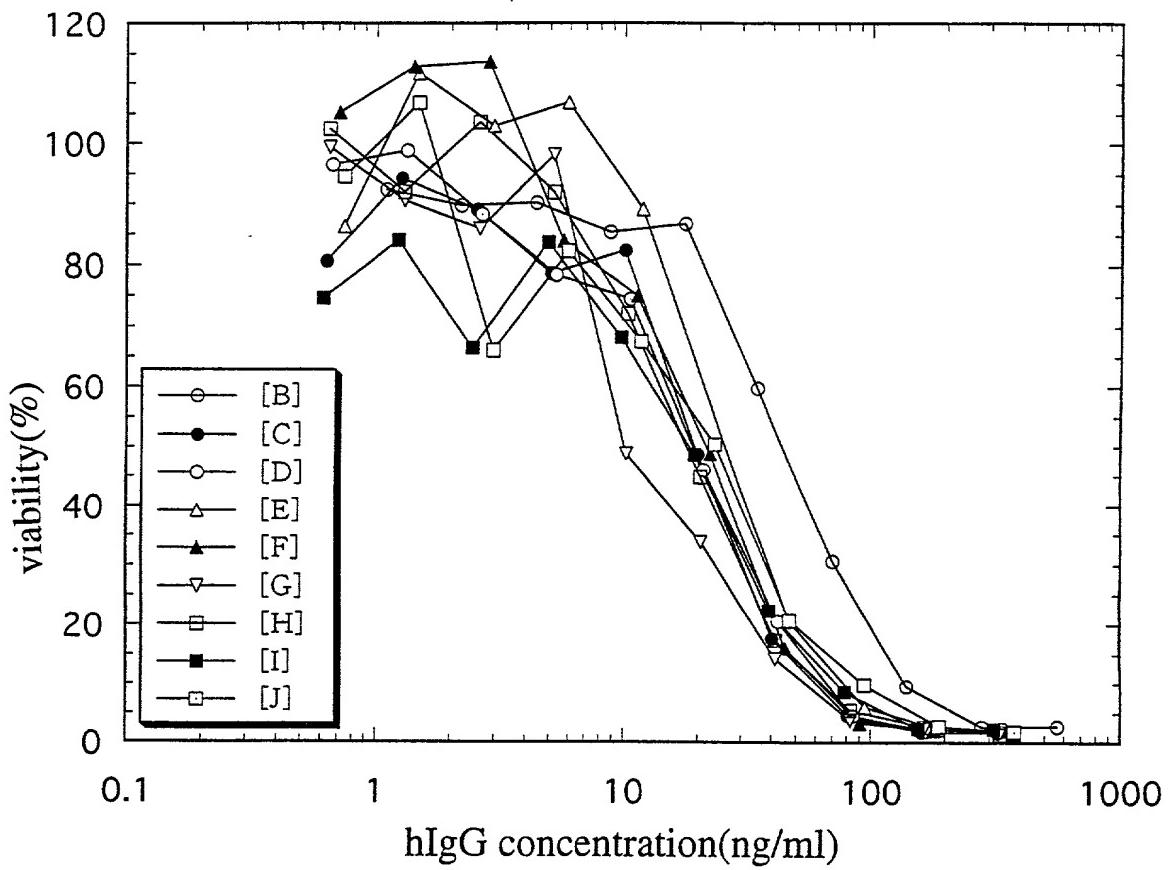


Fig. 66

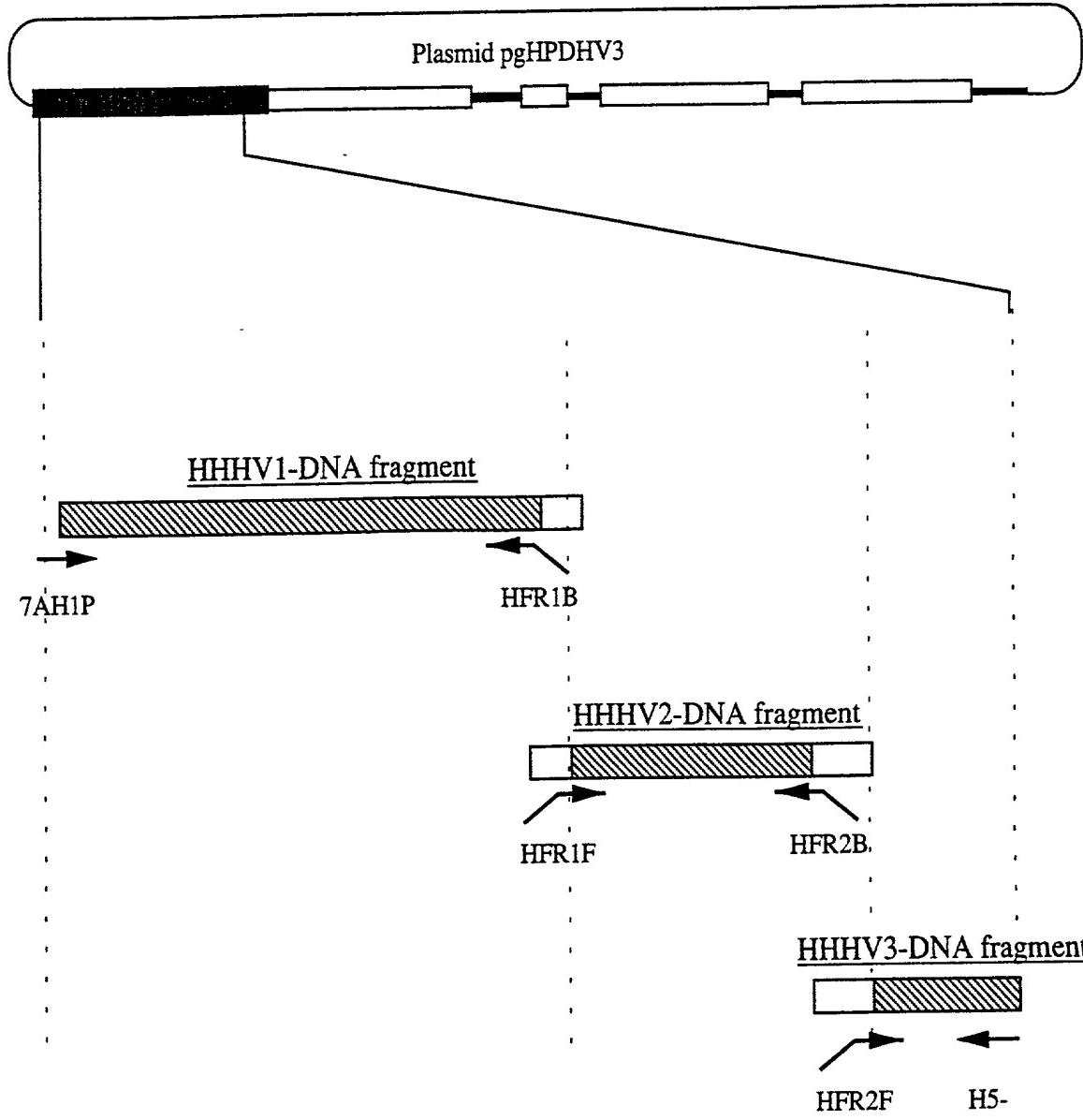
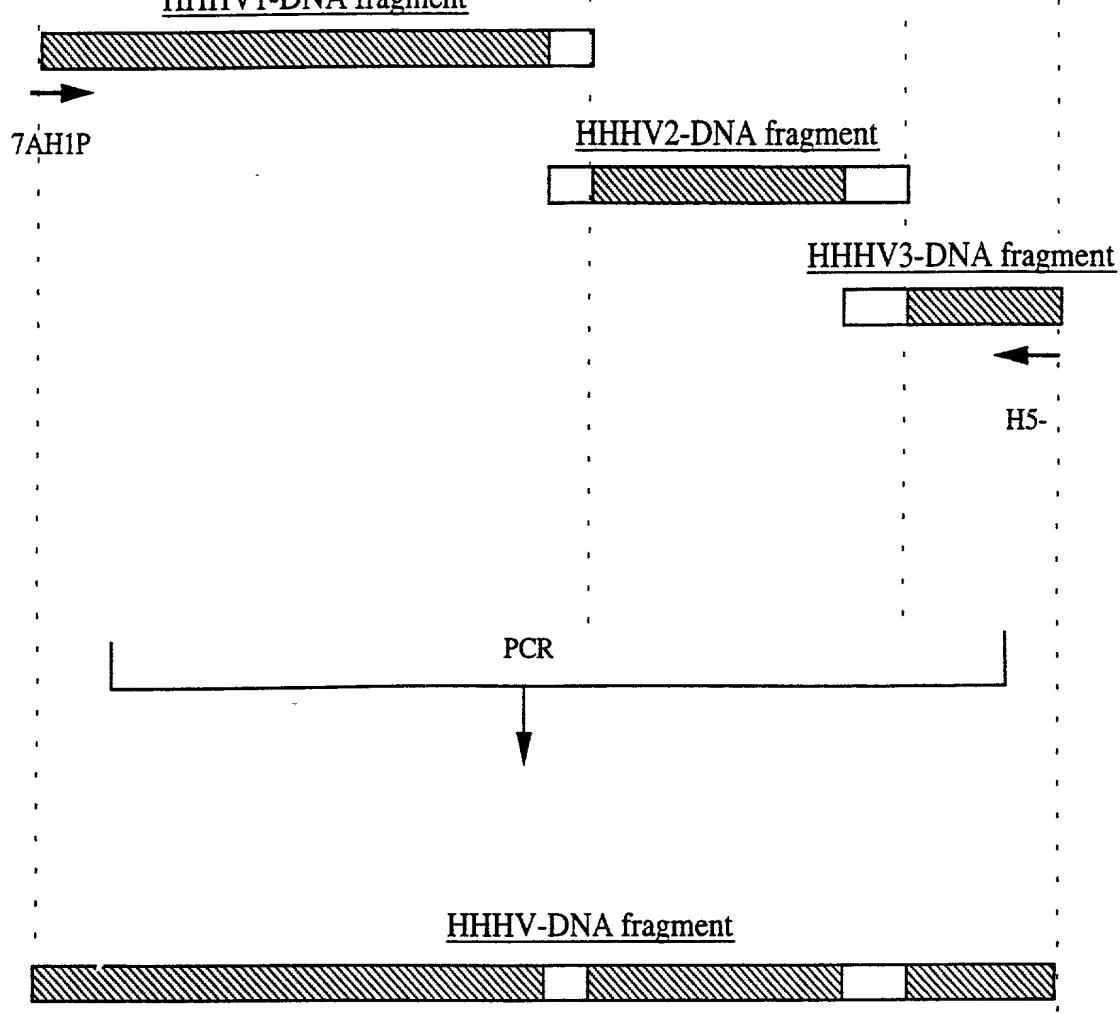


Fig. 67



**Fig. 68**

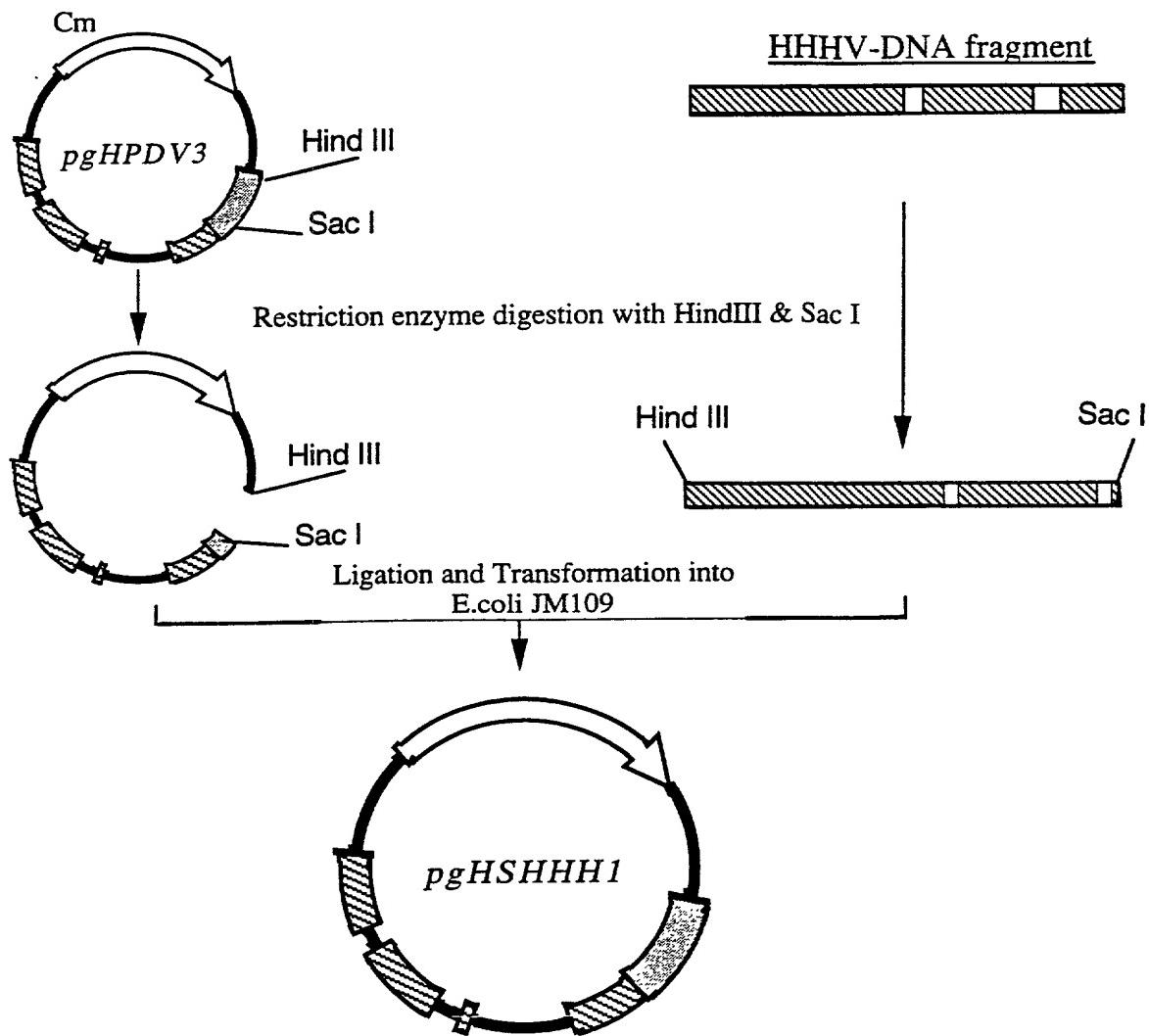


Fig. 69

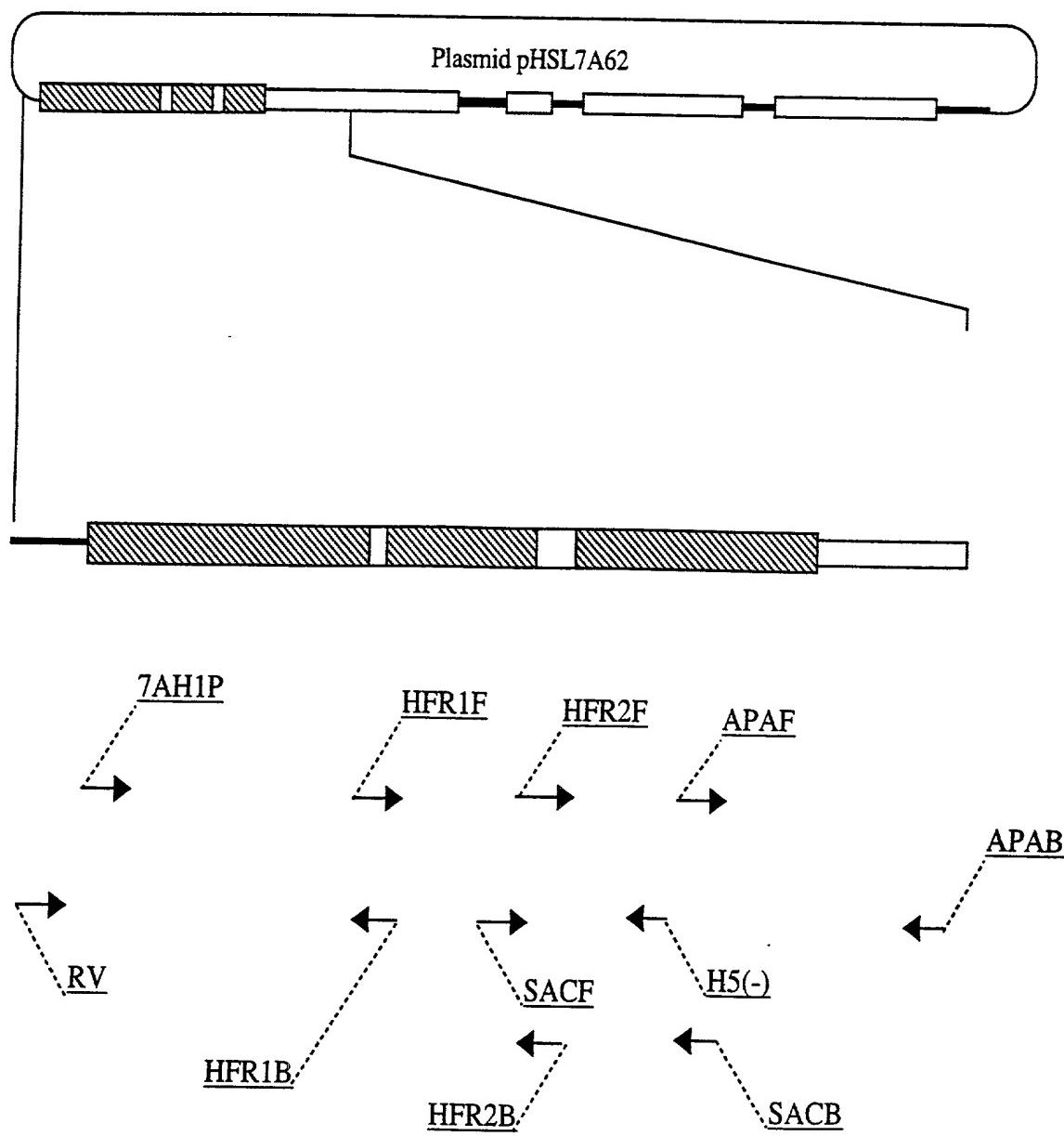


Fig. 70

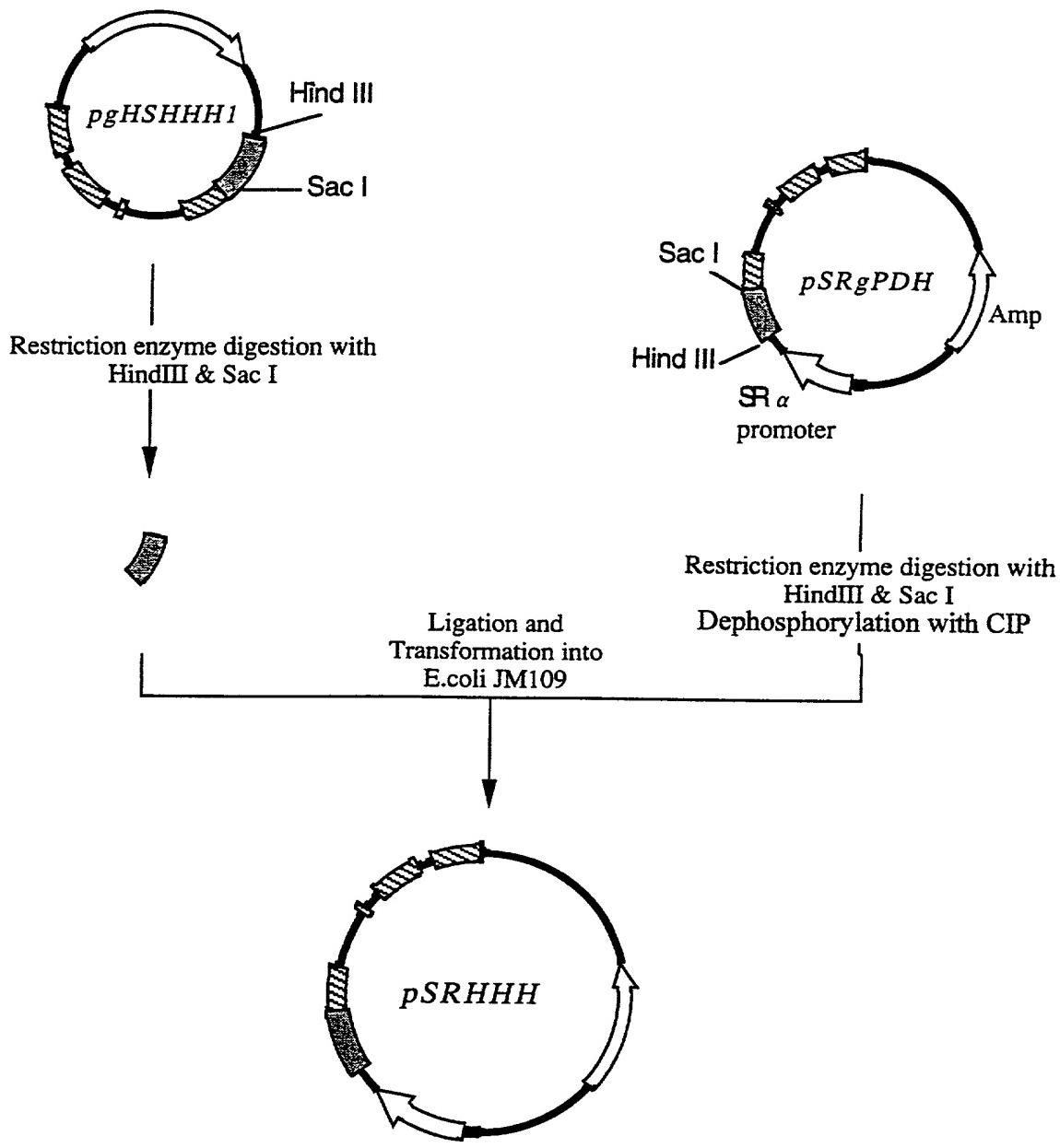


Fig. 71

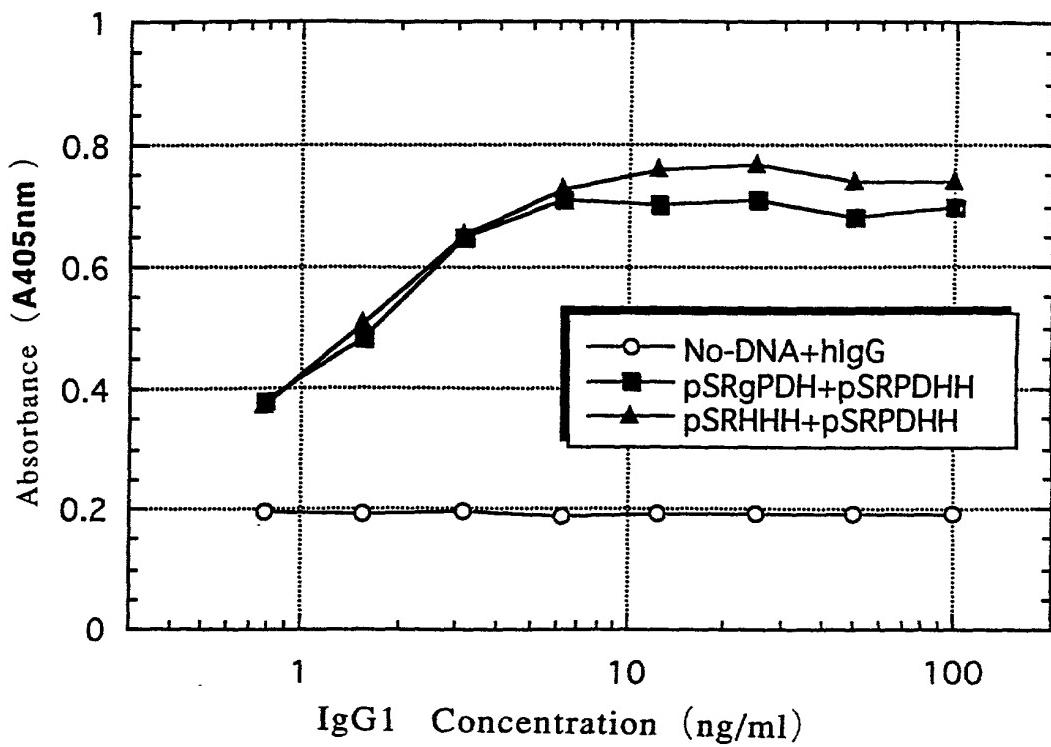


Fig. 72

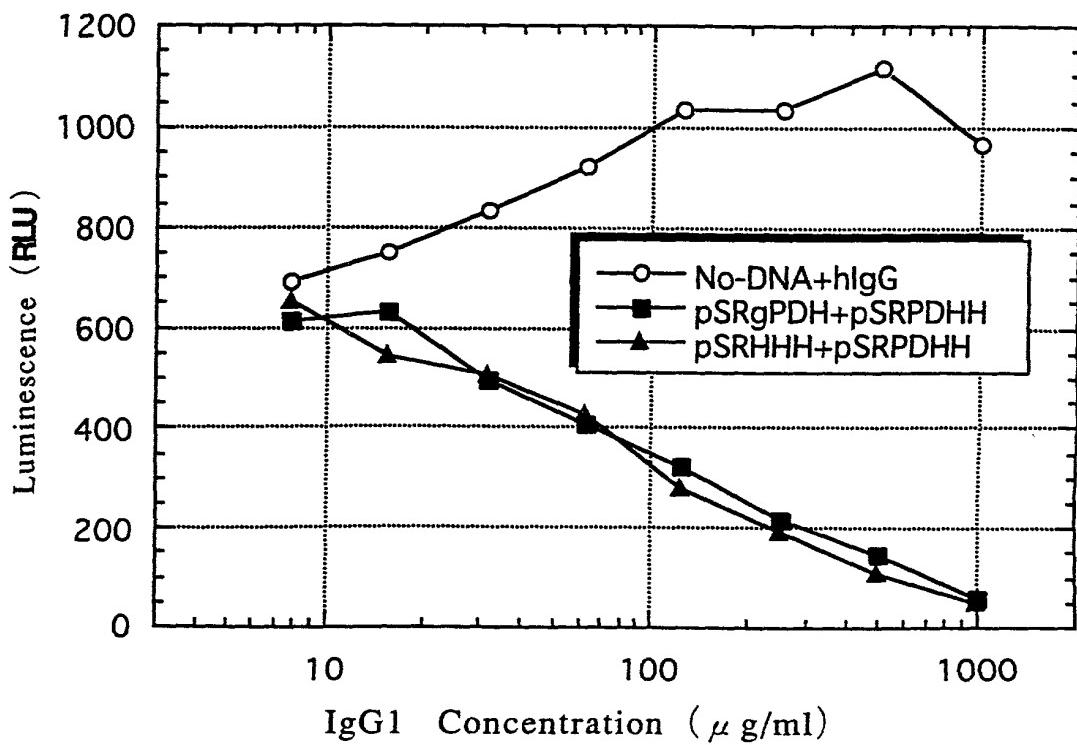


Fig. 73

# APPLICATION FOR UNITED STATES LETTERS PATENT

## CIP DECLARATION AND POWER OF ATTORNEY

As a below named inventor, I declare that:

My residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention entitled:

**Anti-Fas Antibodies**

the specification of which is attached hereto.

I have reviewed and understand the contents of the above-identified specification, including the claims.

I claim priority benefits under 35 USC §119 of: (i) any foreign application(s) for patent or inventor's certificate listed below; or (ii) any United States provisional application(s) listed below; and have also identified below any foreign application(s) for patent or inventor's certificate, or PCT international application having a filing date before that of the application(s) on which priority is claimed.

COUNTRY	APPLICATION NUMBER	DATE (day, month, year)	PRIORITY CLAIMED
Japan	Hei 9-82953	1 April 1997	yes <input checked="" type="checkbox"/> no _____
Japan	Hei 9-169088	25 June 1997	yes <input checked="" type="checkbox"/> no _____
Japan	Hei 9-276064	8 October 1997	yes <input checked="" type="checkbox"/> no _____
Japan	Hei 10-276881	30 September 1998	yes <input checked="" type="checkbox"/> no _____
Japan	Hei 10-276882	30 September 1998	yes <input checked="" type="checkbox"/> no _____

I hereby claim the benefit under 35 USC §120 of the prior application(s) listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in the prior application(s) in a manner provided by the first paragraph of 35 USC §112, I acknowledge the duty to disclose information which is material to the examination of this application including material information which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application in accordance with 37 CFR 1.56(a).

09/053,583  
(Application Serial No.)

April 1, 1998  
(Filing Date)

pending  
(Status)  
(patented, pending, abandoned)

09/408,646  
(Application Serial No.)

September 30, 1999  
(Filing Date)

pending  
(Status)  
(patented, pending, abandoned)

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

I appoint the following attorneys to prosecute this application and to transact all business in the U.S. Patent & Trademark Office connected therewith: Leonard Holtz, Reg. No. 22,974; Herbert Goodman, Reg. No. 17,081; Thomas Langer, Reg. No. 27,264; Marshall J. Chick, Reg. No. 26,853; Richard S. Barth, Reg. No. 28,180; Douglas Holtz, Reg. No. 33,902; and Robert P. Michal, Reg. No. 35,614.

CORRESPONDENCE AND CALLS TO: FRISHAUF, HOLTZ, GOODMAN, LANGER & CHICK, P.C.  
 767 Third Avenue - 25th Floor Tel.: (212) 319-4900  
 New York, New York 10017 Fax.: (212) 319-5101

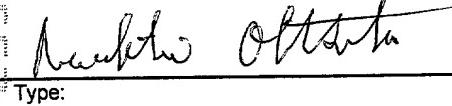
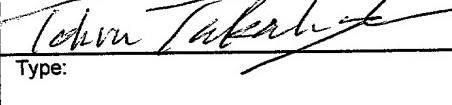
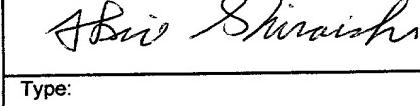
## APPLICATION FOR UNITED STATES LETTERS PATENT

CIP Declaration and Power of Attorney

## INVENTOR:SIGNATURE

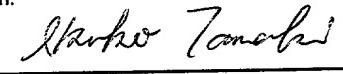
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## RESIDENCE AND POST OFFICE ADDRESS

Sign:  Type: Nobufusa Serizawa	Date: 2 February 2000  Citizen of: Japan	Residence (City & Country): Yokohama-shi, Kanagawa-ken, Japan  Post Office Address: SANKYO COMPANY LIMITED 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo, Japan
Sign:  Type: Kimihisa Ichikawa	Date: 3 February 2000  Citizen of: Japan	Residence (City & Country): Yokohama-shi, Kanagawa-ken, Japan  Post Office Address: SANKYO COMPANY LIMITED 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo, Japan
Sign:  Type: Jun Ohsumi	Date: 2 February 2000  Citizen of: Japan	Residence (City & Country): Kawasaki-shi, Kanagawa-ken, Japan  Post Office Address: SANKYO COMPANY LIMITED 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo, Japan
Sign:  Type: Masahiko Ohtsuki	Date: 1 February 2000  Citizen of: Japan	Residence (City & Country): Yokohama-shi, Kanagawa-ken, Japan  Post Office Address: SANKYO COMPANY LIMITED 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo, Japan
Sign:  Type: Hideyuki Haruyama	Date: 1 February 2000  Citizen of: Japan	Residence (City & Country): Kawagoe-shi, Saitama-ken, Japan  Post Office Address: SANKYO COMPANY LIMITED 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo, Japan
Sign:  Type: Tohru Takahashi	Date: 3 February 2000  Citizen of: Japan	Residence (City & Country): Nerima-ku, Tokyo, Japan  Post Office Address: SANKYO COMPANY LIMITED 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo, Japan
Sign:  Type: Hiroko Yoshida	Date: 2 February 2000  Citizen of: Japan	Residence (City & Country): Nerima-ku, Tokyo, Japan  Post Office Address: SANKYO COMPANY LIMITED 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo, Japan
Sign:  Type: Akio Shiraishi	Date: 1 February 2000  Citizen of: Japan	Residence (City & Country): Shibuya-ku, Tokyo, Japan  Post Office Address: SANKYO COMPANY LIMITED 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo, Japan

**APPLICATION FOR UNITED STATES LETTERS PATENT**  
**CIP Declaration and Power of Attorney**

**INVENTOR:SIGNATURE****DATE****RESIDENCE AND POST OFFICE ADDRESS**

Sign: 	Date: 5 February 2000	Residence (City & Country): Kyoto-shi, Kyoto-fu, Japan Post Office Address: 9-5, Matsuoido-cho, Nishikyo-ku, Kyoto-shi, Kyoto-fu, Japan
Type: Shin Yonehara	Citizen of: Japan	
Sign: 	Date: 1 February 2000	Residence (City & Country): Bunkyo-ku, Tokyo, Japan Post Office Address: SANKYO COMPANY LIMITED 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo, Japan
Type: Kaori Nakahara	Citizen of: Japan	
Sign: 	Date: 2 February 2000	Residence (City & Country): Yokohama-shi, Kanagawa-ken, Japan Post Office Address: SANKYO COMPANY LIMITED 2-58, Hiromachi 1-chome, Shinagawa-ku, Tokyo, Japan
Type: Ikuko Tamaki	Citizen of: Japan	

## CIP Declaration and Power of Attorney

COUNTRY	APPLICATION NO.	FILING DATE	*PATENT OR **PUBLICATION NO.	DATE
Japan (a)	Hei 9-82953	April 1, 1997		
	This application is treated as it has been withdrawn.			
Japan (b)	Hei 9-169088	June 25, 1997		
	This application is treated as it has been withdrawn.			
Japan (c)	Hei 9-276064	October 8, 1997		
	This application is treated as it has been withdrawn.			
Japan	Hei 10-89031	April 1, 1998	** Hei 11-171900	June 29, 1999
	This is an application claiming priority based on the earlier domestic applications (a), (b) & (c).			
Japan (d)	Hei 10-276881	September 30, 1998		
	This application is treated as it has been withdrawn.			
Japan (e)	Hei 10-276882	September 30, 1998		
	This application is treated as it has been withdrawn.			
Japan	Hei 11-275441	September 29, 1999		
	This is an application claiming priority based on the earlier domestic application (d).			
Japan	Hei 11-275440	September 29, 1999		
	This is an application claiming priority based on the earlier domestic application (e).			
Argentina	P 980101481	April 1, 1998		
Argentina	P 990104968	September 30, 1999		
Australia	59701/98	March 30, 1998		
Australia	50161/99	September 28, 1999		
Brazil	PI 9803296-8	April 1, 1998		
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China	98108265.3	April 1, 1998	** CN 1207395A	February 10, 1999
China				
Czech Republic	PV 985-98	April 1, 1998		
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EPC	99307711.4	September 29, 1999		
Hong Kong	99103175	April 1, 1998		
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Hungary	P 9800744	April 1, 1998		
Hungary				
Indonesia	P 980478	March 31, 1998	** 020117A	October 1, 1998
Indonesia	P 990903	September 29, 1999		

## CIP Declaration and Power of Attorney

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India	821/Cal/99	September 30, 1999		
Korea	11477/1998	April 1, 1998		
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Norway	P 981454	March 31, 1998		
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New Zealand	330095	March 31, 1998	* 330095	April 23, 1999
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Philippines	1-1998-00746	March 31, 1998		
Philippines	1-1999-02441	September 29, 1999		
Poland	P 325667	April 1, 1998		
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Russia	98105631	March 31, 1998		
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Saudi Arabia	98190114	June 3, 1998		
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South Africa	98/2719	March 31, 1998		
South Africa				
Thailand	43086	March 31, 1998		
Thailand	53051	September 29, 1999		
Taiwan	87104806	March 31, 1998		
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Turkey	1998/604	April 1, 1998		
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U. S. A.	09/053,583	April 1, 1998		
U. S. A.	09/408,646	September 30, 1999		
Venezuela	637-98	April 1, 1998		
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Nakahara, Kaori  
Tamaki, Ikuko  
Takahashi, Tohru

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<210> 4  
<211> 12  
<212> PRT  
<213> Mus musculus

<400> 4  
Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp Val  
1 5 10

<210> 5  
<211> 15  
<212> PRT  
<213> Mus musculus

<400> 5  
Lys Ala Ser Gln Ser Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn  
1 5 10 15

<210> 6  
<211> 7  
<212> PRT  
<213> Mus musculus

<400> 6  
Ala Ala Ser Asn Leu Glu Ser  
1 5

<210> 7  
<211> 9  
<212> PRT  
<213> Mus musculus

<400> 7  
Gln Gln Ser Asn Glu Asp Pro Arg Thr  
1 5

<210> 8  
<211> 1392  
<212> DNA  
<213> Mus musculus

<220>  
<221> CDS  
<222> (1)..(1392)

<220>  
<221> mat peptide  
<222> (58)..(1392)

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<220>
<221> sig peptide
<222> (1)..(57)

<400> 8
atg gga tgg agc tgt atc atc ctc ttc ttg gta gca aca gct aca ggt 48
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
-15 -10 -5

gtc cat tct cag gtc caa ctg cag cag cct ggg gct gag ctt gtg aag 96
Val His Ser Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys
-1 1 5 10

cct ggg gct tca gtg aag ctg tcc tgc aag gct tct ggc tac acc ttc 144
Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe
15 20 25

acc agc tac tgg atg cag tgg gta aaa cag agg cct gga cag ggc ctt 192
Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
30 35 40 45

gag tgg atc gga gag att gat cct tct gat agc tat act aac tac aat 240
Glu Trp Ile Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn
50 55 60

caa aag ttc aag ggc aag gcc aca ttg act gta gac aca tcc tcc agc 288
Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser
65 70 75

aca gcc tac atg cag ctc agc agc ctg aca tct gag gac tct gcg gtc 336
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
80 85 90

tat tac tgt gca aga aat agg gac tat agt aac aac tgg tac ttc gat 384
Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp
95 100 105

gtc tgg ggc aca ggg acc acg gtc acc gtc tcc tca gcc aaa acg aca 432
Val Trp Gly Thr Gly Thr Val Thr Val Ser Ser Ala Lys Thr Thr
110 115 120 125

ccc cca tct gtc tat cca ctg gcc cct gga tct gct gcc caa act aac 480
Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn
130 135 140

tcc atg gtg acc ctg gga tgc ctg gtc aag ggc tat ttc cct gag cca 528
Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro
145 150 155

gtg aca gtg acc tgg aac tct gga tcc ctg tcc agc ggt gtg cac acc 576
Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr
160 165 170

ttc cca gct gtc ctg cag tct gac ctc tac act ctg agc agc tca gtg 624

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aat ggg cag cca gcg gag aac tac aag aac act cag ccc atc atg aac		1248
Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asn		
385	390	395
acg aat ggc tct tac ttc gtc tac agc aag ctc aat gtg cag aag agc		1296
Thr Asn Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser		
400	405	410
aac tgg gag gca gga aat act ttc acc tgc tct gtg tta cat gag ggc		1344
Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly		
415	420	425
ctg cac aac cac cat act gag aag agc ctc tcc cac tct cct ggt aaa		1392
Leu His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys		
430	435	440

<210> 9  
<211> 464  
<212> PRT  
<213> Mus musculus

<400> 9		
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly		
-15	-10	-5
Val His Ser Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Lys		
-1 1	5	10
Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe		
15	20	25
Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Arg Pro Gly Gln Gly Leu		
30	35	40
Glu Trp Ile Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn		
50	55	60
Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ser Ser		
65	70	75
Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val		
80	85	90
Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp		
95	100	105
Val Trp Gly Thr Gly Thr Thr Val Thr Val Ser Ser Ala Lys Thr Thr		
110	115	120
Pro Pro Ser Val Tyr Pro Leu Ala Pro Gly Ser Ala Ala Gln Thr Asn		
130	135	140

Ser Met Val Thr Leu Gly Cys Leu Val Lys Gly Tyr Phe Pro Glu Pro  
145 150 155

Val Thr Val Thr Trp Asn Ser Gly Ser Leu Ser Ser Gly Val His Thr  
160 165 170

Phe Pro Ala Val Leu Gln Ser Asp Leu Tyr Thr Leu Ser Ser Ser Val  
175 180 185

Thr Val Pro Ser Ser Thr Trp Pro Ser Gln Thr Val Thr Cys Asn Val  
190 195 200 205

Ala His Pro Ala Ser Ser Thr Lys Val Asp Lys Lys Ile Val Pro Arg  
210 215 220

Asp Cys Gly Cys Lys Pro Cys Ile Cys Thr Val Pro Glu Val Ser Ser  
225 230 235

Val Phe Ile Phe Pro Pro Lys Pro Lys Asp Val Leu Thr Ile Thr Leu  
240 245 250

Thr Pro Lys Val Thr Cys Val Val Val Asp Ile Ser Lys Asp Asp Pro  
255 260 265

Glu Val Gln Phe Ser Trp Phe Val Asp Asp Val Glu Val His Thr Ala  
270 275 280 285

Gln Thr Gln Pro Arg Glu Glu Gln Phe Asn Ser Thr Phe Arg Ser Val  
290 295 300

Ser Glu Leu Pro Ile Met His Gln Asn Trp Leu Asn Gly Lys Glu Phe  
305 310 315

Lys Cys Arg Val Asn Ser Ala Ala Phe Pro Ala Pro Ile Glu Lys Thr  
320 325 330

Ile Ser Lys Thr Lys Gly Arg Pro Lys Ala Pro Gln Val Tyr Thr Ile  
335 340 345

Pro Pro Pro Lys Glu Gln Met Ala Lys Asp Lys Val Ser Leu Thr Cys  
350 355 360 365

Met Ile Thr Asp Phe Phe Pro Glu Asp Ile Thr Val Glu Trp Gln Trp  
370 375 380

Asn Gly Gln Pro Ala Glu Asn Tyr Lys Asn Thr Gln Pro Ile Met Asn  
385 390 395

Thr Asn Gly Ser Tyr Phe Val Tyr Ser Lys Leu Asn Val Gln Lys Ser  
400 405 410

Asn Trp Glu Ala Gly Asn Thr Phe Thr Cys Ser Val Leu His Glu Gly  
415 420 425

Leu His Asn His His Thr Glu Lys Ser Leu Ser His Ser Pro Gly Lys  
 430                  435                  440                  445

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<210> 10  
<211> 714  
<212> DNA  
<213> Mus musculus
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<220>  
<221> CDS  
<222> (1) . . (714)

<220>  
<221> mat peptide  
<222> (61)..(714)

<220>  
<221> sig peptide  
<222> (1)..(60)

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<400> 10
atg gag aca gac aca atc ctg cta tgg gtg atg atg ctc tgg att cca      48
Met Glu Thr Asp Thr Ile Leu Leu Trp Val Met Met Leu Trp Ile Pro
-20          -15           -10           -5

```

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ggc tcc act ggt gac att gtg ctg acc caa tct cca gct tct ttg gct 96
Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala
      -1   1           5           10

```

gtg tct cta ggg cag agg gcc acc atc tcc tgc aag gcc agc caa agt 144  
 Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser  
                  15                 20                 25

gtt gat tat gat ggt gat agt tat atg aac tgg tac caa cag aaa cca 192  
 Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro  
           30             35             40

gga cag cca ccc aaa ctc ctc atc tat gct gca tcc aat cta gaa tct 240  
 Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
   45              50              55              60

ggg atc cca gcc agg ttt agt ggc agt ggg tct ggg aca gac ttc acc 288  
 Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
                   65                 70                 75

ctc aac atc cat cct gtg gag gag gag gat gct gca acc tat tac tgt 336  
 Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys  
                   80                  85                  90

cag caa agt aat gag gat cct cg<sup>g</sup> acg ttc ggt gga ggc acc aag ctg 384  
 Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gly Thr Lys Leu  
           95             100             105

gaa atc aaa cg	gct gat gct gca cca act gta tcc atc ttc cca cca	432	
Glu Ile Lys Arg Ala Asp	Ala Ala Pro Thr Val Ser Ile Phe Pro Pro		
110	115	120	
tcc agt gag cag tta aca tct gga ggt gcc tca gtc gtg tgc ttc ttg	Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu	480	
125	130	135	140
aac aac ttc tac ccc aaa gac atc aat gtc aag tgg aag att gat ggc	Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly	528	
145	150	155	
agt gaa cga caa aat ggc gtc ctg aac agt tgg act gat cag gac agc	Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser	576	
160	165	170	
aaa gac agc acc tac agc atg agc agc acc ctc acg ttg acc aag gac	Lys Asp Ser Thr Tyr Ser Met Ser Thr Leu Thr Leu Thr Lys Asp	624	
175	180	185	
gag tat gaa cga cat aac agc tat acc tgt gag gcc act cac aag aca	Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr	672	
190	195	200	
tca act tca ccc att gtc aag agc ttc aac agg aat gag tgt	Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys	714	
205	210	215	
<210> 11			
<211> 238			
<212> PRT			
<213> Mus musculus			
<400> 11			
Met Glu Thr Asp Thr Ile Leu Leu Trp Val Met Met Leu Trp Ile Pro			
-20	-15	-10	-5
Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala			
-1 1	5	10	
Val Ser Leu Gly Gln Arg Ala Thr Ile Ser Cys Lys Ala Ser Gln Ser			
15	20	25	
Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro			
30	35	40	
Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser			
45	50	55	60
Gly Ile Pro Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr			
65	70	75	

Leu Asn Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys  
80 85 90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gly Gly Thr Lys Leu  
95 100 105

Glu Ile Lys Arg Ala Asp Ala Ala Pro Thr Val Ser Ile Phe Pro Pro  
110 115 120

Ser Ser Glu Gln Leu Thr Ser Gly Gly Ala Ser Val Val Cys Phe Leu  
125 130 135 140

Asn Asn Phe Tyr Pro Lys Asp Ile Asn Val Lys Trp Lys Ile Asp Gly  
145 150 155

Ser Glu Arg Gln Asn Gly Val Leu Asn Ser Trp Thr Asp Gln Asp Ser  
160 165 170

Lys Asp Ser Thr Tyr Ser Met Ser Ser Thr Leu Thr Leu Thr Lys Asp  
175 180 185

Glu Tyr Glu Arg His Asn Ser Tyr Thr Cys Glu Ala Thr His Lys Thr  
190 195 200

Ser Thr Ser Pro Ile Val Lys Ser Phe Asn Arg Asn Glu Cys  
205 210 215

<210> 12

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to  
amplify a DNA encoding the extracellular region of  
human Fas antigen

<400> 12

gggaaattcc agtacggagt tggggaaagct cttt

34

<210> 13

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to  
amplify a DNA encoding the extracellular region of  
human Fas antigen

<400> 13

gtttcttctg cctctgtcac caagtttagat ctgga

35

<210> 14  
<211> 35  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a DNA encoding the extracellular region of mouse IL-3 receptor

<400> 14 tccagatcta acttggtgac agaggcagaa gaaac 35

<210> 15  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a DNA encoding the extracellular region of mouse IL-3 receptor

<400> 15 ccctctagac gcgtcacgtg ggcacatcac 28

<210> 16  
<211> 11  
<212> PRT  
<213> Mus musculus

<220>  
<221> Unsure  
<222> 2  
<223> Unidentified amino acid

<400> 16  
Gln Xaa Gln Leu Gln Gln Pro Gly Ala Glu Leu  
1 5 10

<210> 17  
<211> 22  
<212> PRT  
<213> Mus musculus

<400> 17  
Asp Ile Val Leu Thr Gln Ser Pro Ala Ser Leu Ala Val Ser Leu Gly  
1 5 10 15

Gln Arg Ala Thr Ile Ser  
20

<210> 18  
<211> 19  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a DNA encoding mouse immunoglobulin heavy  
chain gamma 1 subtype 2b  
  
<400> 18  
gacctcacca tggatgga 19  
  
<210> 19  
<211> 20  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a DNA encoding mouse immunoglobulin heavy  
chain gamma 1 subtype 2b  
  
<400> 19  
tttaccagga gagtggaga 20  
  
<210> 20  
<211> 20  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a DNA encoding mouse immunoglobulin light  
chain kappa subtype 3  
  
<400> 20  
aagaaggcatc ctctcatcta 20  
  
<210> 21  
<211> 20  
<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a DNA encoding mouse immunoglobulin light

chain kappa subtype 3

<400> 21  
acactcattc ctgttgaagc

20

<210> 22  
<211> 28  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Adaptor primer  
to subclone a cDNA encoding the heavy chain of  
anti-human Fas antibody HFE7A

<400> 22  
gggaaattcg acctcaccat gggatgga

28

<210> 23  
<211> 32  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Adaptor primer  
to subclone a cDNA encoding the heavy chain of  
anti-human Fas antibody HFE7A

<400> 23  
gggtcttagac tatttaccag gagagtggga ga

32

<210> 24  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Adaptor primer  
to subclone a cDNA encoding the light chain of  
anti-human Fas antibody HFE7A

<400> 24  
gggaaattca agaagcatcc tctcatcta

29

<210> 25  
<211> 37  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Adaptor primer  
to subclone a cDNA encoding the light chain of  
anti-human Fas antibody HFE7A

<400> 25  
ggggcggccg cttactaaca ctcattcctg ttgaagc

37

<210> 26  
<211> 19  
<212> PRT  
<213> Homo sapiens

<400> 26  
Arg Leu Ser Ser Lys Ser Val Asn Ala Gln Val Thr Asp Ile Asn Ser  
1 5 10 15  
Lys Gly Leu

<210> 27  
<211> 19  
<212> PRT  
<213> Homo sapiens

<400> 27  
Val Thr Asp Ile Asn Ser Lys Gly Leu Glu Leu Arg Lys Thr Val Thr  
1 5 10 15  
Thr Val Glu

<210> 28  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 28  
Glu Leu Arg Lys Thr Val Thr Val Glu Thr Gln Asn Leu Glu Gly  
1 5 10 15  
Leu His His Asp  
20

<210> 29  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 29

Thr Gln Asn Leu Glu Gly Leu His His Asp Gly Gln Phe Cys His Lys  
1 5 10 15

Pro Cys Pro Pro  
20

<210> 30  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 30  
Gly Gln Phe Cys His Lys Pro Cys Pro Pro Gly Glu Arg Lys Ala Arg  
1 5 10 15

Asp Cys Thr Val  
20

<210> 31  
<211> 21  
<212> PRT  
<213> Homo sapiens

<400> 31  
Gly Glu Arg Lys Ala Arg Asp Cys Thr Val Asn Gly Asp Glu Pro Asp  
1 5 10 15

Cys Val Pro Cys Gln  
20

<210> 32  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 32  
Asn Gly Asp Glu Pro Asp Cys Val Pro Cys Gln Glu Gly Lys Glu Tyr  
1 5 10 15

Thr Asp Lys Ala  
20

<210> 33  
<211> 19  
<212> PRT  
<213> Homo sapiens

<400> 33  
Glu Gly Lys Glu Tyr Thr Asp Lys Ala His Phe Ser Ser Lys Cys Arg  
1 5 10 15

Arg Cys Arg

<210> 34

<211> 20

<212> PRT

<213> Homo sapiens

<400> 34

His Phe Ser Ser Lys Cys Arg Arg Cys Arg Leu Cys Asp Glu Gly His  
1 5 10 15

Gly Leu Glu Val

20

<210> 35

<211> 20

<212> PRT

<213> Homo sapiens

<400> 35

Leu Cys Asp Glu Gly His Gly Leu Glu Val Glu Ile Asn Cys Thr Arg  
1 5 10 15

Thr Gln Asn Thr

20

<210> 36

<211> 20

<212> PRT

<213> Homo sapiens

<400> 36

Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr Lys Cys Arg Cys Lys Pro  
1 5 10 15

Asn Phe Phe Cys

20

<210> 37

<211> 20

<212> PRT

<213> Homo sapiens

<400> 37

Lys Cys Arg Cys Lys Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu  
1 5 10 15

His Cys Asp Pro

20

<210> 38  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 38  
Asn Ser Thr Val Cys Glu His Cys Asp Pro Cys Thr Lys Cys Glu His  
1 5 10 15

Gly Ile Ile Lys  
20

<210> 39  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 39  
Cys Thr Lys Cys Glu His Gly Ile Ile Lys Glu Cys Thr Leu Thr Ser  
1 5 10 15

Asn Thr Lys Cys  
20

<210> 40  
<211> 18  
<212> PRT  
<213> Homo sapiens

<400> 40  
Glu Cys Thr Leu Thr Ser Asn Thr Lys Cys Lys Glu Glu Gly Ser Arg  
1 5 10 15

Ser Asn

<210> 41  
<211> 20  
<212> PRT  
<213> Homo sapiens

<400> 41  
Ser Ser Gly Lys Tyr Glu Gly Gly Asn Ile Tyr Thr Lys Lys Glu Ala  
1 5 10 15

Phe Asn Val Glu  
20

<210> 42

<211> 10  
<212> PRT  
<213> Homo sapiens

<400> 42  
His Gly Leu Glu Val Glu Ile Asn Cys Thr  
1 5 10

<210> 43  
<211> 10  
<212> PRT  
<213> Homo sapiens

<400> 43  
Glu Ile Asn Cys Thr Arg Thr Gln Asn Thr  
1 5 10

<210> 44  
<211> 10  
<212> PRT  
<213> Homo sapiens

<400> 44  
Lys Cys Arg Cys Lys Pro Asn Phe Phe Cys  
1 5 10

<210> 45  
<211> 14  
<212> PRT  
<213> Homo sapiens

<400> 45  
Pro Asn Phe Phe Cys Asn Ser Thr Val Cys Glu His Cys Asp  
1 5 10

<210> 46  
<211> 10  
<212> PRT  
<213> Homo sapiens

<400> 46  
Gly Lys Ile Ala Ser Cys Leu Asn Asp Asn  
1 5 10

<210> 47  
<211> 34  
<212> DNA  
<213> Homo sapiens

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<400> 47
gcgaattctg ccttgactga tcagagttc ctca

<210> 48
<211> 32
<212> DNA
<213> Homo sapiens

<400> 48
gctcttagatg aggtgaaaga tgagctggag ga 32

<210> 49
<211> 768
<212> DNA
<213> Artificial Sequence

<220>
<221> CDS
<222> (40)..(753)

<220>
<221> mat peptide
<222> (100)..(753)

<220>
<221> sig peptide
<222> (40)..(99)

<220>
<223> Description of Artificial Sequence: Designed DNA
encoding the light chain of humanized anti-human
Fas antibody

<400> 49
cccaagctta agaaggcatcc tctcatctag ttctcagag atg gag aca gac aca 54
Met Glu Thr Asp Thr
-20

atc ctg cta tgg gtg ctg ctc tgg gtt cca ggc tcc act ggt gac
Ile Leu Leu Trp Val Leu Leu Trp Val Pro Gly Ser Thr Gly Asp 102
-15 -10 -5 -1 1

att gtg ctc acc caa tct cca ggt act ttg tct ctg tct cca ggg gag
Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu 150
5 10 15

agg gcc acc ctc tcc tgc aag gcc agc caa agt gtt gat tat gat ggt
Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp Gly 198
20 25 30

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gat agt tat atg aac tgg tac caa cag aaa cca gga cag gca ccc aga	246		
Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg			
35	40	45	
ctc ctc atc tat gct gca tcc aat ctc gaa tct ggg atc cca gac agg	294		
Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Ile Pro Asp Arg			
50	55	60	65
ttt agt ggc agt ggg tct ggg aca gac ttc acc ctc acc atc tct cgt	342		
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Arg			
70	75	80	
ctg gag ccg gcg gat ttt gca gtc tat tac tgt cag caa agt aat gag	390		
Leu Glu Pro Ala Asp Phe Ala Val Tyr Tyr Cys Gln Gln Ser Asn Glu			
85	90	95	
gat cct cg <sup>g</sup> acg ttc ggt caa ggc acc agg ctg gaa atc aaa cg <sup>g</sup> act	438		
Asp Pro Arg Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr			
100	105	110	
gtg gct gca cca tct gtc ttc atc ttc cc <sup>g</sup> cca tct gat gag cag ttg	486		
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu			
115	120	125	
aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc	534		
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro			
130	135	140	145
aga gag gcc aaa gta cag tgg aaa gtg gat aac gcc ctc caa tcg ggt	582		
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly			
150	155	160	
aac tcc cag gag agt gtc aca qag cag gac agc aag gac agc acc tac	630		
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr			
165	170	175	
agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac	678		
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His			
180	185	190	
aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc	726		
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val			
195	200	205	
aca aag agc ttc aac agg gga gag tgt tagtaagaat tcggg	768		
Thr Lys Ser Phe Asn Arg Gly Glu Cys			
210	215		

<210> 50  
<211> 238  
<212> PRT  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed light chain of humanized anti-Fas antibody

<400> 50

Met Glu Thr Asp Thr Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
-20 -15 -10 -5

Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser  
-1 1 5 10

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser  
15 20 25

Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro  
30 35 40

Gly Gln Ala Pro Arg Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
45 50 55 60

Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr  
65 70 75

Leu Thr Ile Ser Arg Leu Glu Pro Ala Asp Phe Ala Val Tyr Tyr Cys  
80 85 90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Arg Leu  
95 100 105

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro  
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
125 130 135 140

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn  
145 150 155

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
160 165 170

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
175 180 185

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
190 195 200

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
205 210 215

<210> 51

<211> 768

<212> DNA  
<213> Artificial Sequence

<220>  
<221> CDS  
<222> (40)..(753)

<220>  
<221> mat peptide  
<222> (100)..(753)

<220>  
<221> sig peptide  
<222> (40)..(99)

<220>

<223> Description of Artificial Sequence: Designed DNA encoding the light chain of humanized anti-human Fas antibody

<400> 51  
cccaagctta agaaggcatcc tctcatctag ttctcagag atg gag aca gac aca 54  
Met Glu Thr Asp Thr  
-20

atc ctg cta tgg gtg ctg ctg tgg gtt cca ggc tcc act ggt gac 102  
Ile Leu Leu Trp Val Leu Leu Trp Val Pro Gly Ser Thr Gly Asp  
-15 -10 -5 -1 1

att gtg ctc acc caa tct cca ggt act ttg tct ctg tct cca ggg gag 150  
Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu  
5 10 15

agg gcc acc ctc tcc tgc aag gcc agc caa agt gtt gat tat gat ggt 198  
Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp Gly  
20 25 30

gat agt tat atg aac tgg tac caa cag aaa cca gga cag gca ccc aga 246  
Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg  
35 40 45

ctc ctc atc tat gct gca tcc aat ctc gaa tct ggg atc cca gac agg 294  
Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Ile Pro Asp Arg  
50 55 60 65

ttt agt ggc agt ggg tct ggg aca gac ttc acc ctc acc atc cat cct 342  
Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile His Pro  
70 75 80

gtg gag gag gag gat gct gca acc tat tac tgt cag caa agt aat gag 390  
Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu  
85 90 95

gat cct cg <sup>g</sup> acg ttc ggt caa ggc acc agg ctg gaa atc aaa cg <sup>g</sup> act	438
Asp Pro Arg Thr Phe Gly Gln Gly Thr Arg Leu Glu Ile Lys Arg Thr	
100 105 110	
gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag ttg	486
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu	
115 120 125	
aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc	534
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro	
130 135 140 145	
aga gag gcc aaa gta cag tgg aaa gtg gat aac gcc ctc caa tcg ggt	582
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly	
150 155 160	
aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc tac	630
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr	
165 170 175	
agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac	678
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His	
180 185 190	
aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc	726
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val	
195 200 205	
aca aag agc ttc aac agg gga gag tgt tagtaagaat tcggg	768
Thr Lys Ser Phe Asn Arg Gly Glu Cys	
210 215	
<210> 52	
<211> 238	
<212> PRT	
<213> Artificial Sequence	
<220>	
<223> Description of Artificial Sequence: Designed light	
chain of humanized anti-Fas antibody	
<400> 52	
Met Glu Thr Asp Thr Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro	
-20 -15 -10 -5	
Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser	
-1 1 5 10	
Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser	
15 20 25	
Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro	
30 35 40	

Gly Gln Ala Pro Arg Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
45 50 55 60

Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr  
65 70 75

Leu Thr Ile His Pro Val Glu Glu Asp Ala Ala Thr Tyr Tyr Cys  
80 85 90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Arg Leu  
95 100 105

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro  
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
125 130 135 140

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn  
145 150 155

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
160 165 170

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
175 180 185

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
190 195 200

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
205 210 215

<210> 53  
<211> 768  
<212> DNA  
<213> Artificial Sequence

<220>  
<221> CDS  
<222> (40)..(753)

<220>  
<221> mat peptide  
<222> (100)..(753)

<220>  
<221> sig peptide  
<222> (40)..(99)

<220>  
<223> Description of Artificial Sequence: Designed DNA  
encoding the light chain of humanized anti-human

Fas antibody

<400>	53															
cccaagctta	agaagcatcc	tctcatctag	ttctcagag	atg	gag	aca	gac	aca	54							
				Met	Glu	Thr	Asp	Thr								
									-20							
atc	ctg	cta	tgg	gtg	ctg	ctc	tgg	gtt	cca	ggc	tcc	act	ggt	gac	102	
Ile	Leu	Leu	Trp	Val	Leu	Leu	Leu	Trp	Val	Pro	Gly	Ser	Thr	Gly	Asp	
-15				-10					-5					-1	1	
att	gtg	ctc	acc	caa	tct	cca	ggt	act	ttg	tct	ctg	tct	cca	ggg	gag	150
Ile	Val	Leu	Thr	Gln	Ser	Pro	Gly	Thr	Leu	Ser	Leu	Ser	Pro	Gly	Glu	
									5	10				15		
agg	gcc	acc	ctc	tcc	tgc	aag	gcc	agc	caa	agt	gtt	gat	tat	gat	ggt	198
Arg	Ala	Thr	Leu	Ser	Cys	Lys	Ala	Ser	Gln	Ser	Val	Asp	Tyr	Asp	Gly	
									20	25				30		
gat	agt	tat	atg	aac	tgg	tac	caa	cag	aaa	cca	gga	cag	cca	ccc	aaa	246
Asp	Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Pro	Pro	Lys	
									35	40				45		
ctc	ctc	atc	tat	gct	gca	tcc	aat	ctc	gaa	tct	ggg	atc	cca	gac	agg	294
Leu	Leu	Ile	Tyr	Ala	Ala	Ser	Asn	Leu	Glu	Ser	Gly	Ile	Pro	Asp	Arg	
									50	55				65		
ttt	agt	ggc	agt	ggg	tct	ggg	aca	gac	ttc	acc	ctc	acc	atc	cat	cct	342
Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	His	Pro	
									70	75				80		
gtg	gag	gag	gag	gat	gct	gca	acc	tat	tac	tgt	cag	caa	agt	aat	gag	390
Val	Glu	Glu	Glu	Asp	Ala	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Asn	Glu	
									85	90				95		
gat	cct	cg	ac	tt	gg	ca	gg	ac	gg	ct	gaa	at	aa	cg	ac	438
Asp	Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Arg	Leu	Glu	Ile	Lys	Arg	Thr	
									100	105				110		
gtg	gct	gca	cc	tct	gtc	tt	at	tt	cc	cc	tct	gat	gag	cag	tt	486
Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	
									115	120				125		
aaa	tct	gga	act	gcc	tct	gtt	gt	tg	tg	ct	ct	aat	aa	tt	tt	534
Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	
									130	135				145		
aga	gag	gcc	aaa	gta	cag	tgg	aaa	gt	gat	aa	gcc	ct	caa	tc	gg	582
Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	
									150	155				160		
aac	tcc	cag	gag	agt	gtc	aca	gag	cag	gac	agc	aag	gac	agc	acc	tac	630
Asn	Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	
									165	170				175		

agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac 678  
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His  
180 185 190

aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc 726  
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val  
195 200 205

aca aag agc ttc aac agg gga gag tgt tagtaagaat tcggg 768  
Thr Lys Ser Phe Asn Arg Gly Glu Cys  
210 215

<210> 54  
<211> 238  
<212> PRT  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Designed light  
chain of humanized anti-Fas antibody

<400> 54  
Met Glu Thr Asp Thr Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
-20 -15 -10 -5

Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser  
-1 1 5 10

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser  
15 20 25

Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro  
30 35 40

Gly Gln Pro Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
45 50 55 60

Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Thr Asp Phe Thr  
65 70 75

Leu Thr Ile His Pro Val Glu Glu Asp Ala Ala Thr Tyr Tyr Cys  
80 85 90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Arg Leu  
95 100 105

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro  
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
125 130 135 140

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn  
145 150 155

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
160 165 170

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
175 180 185

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
190 195 200

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
205 210 215

<210> 55

<211> 34

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the light  
chain of humanized anti-Fas antibody

<400> 55

cccaagctta agaaggatcc tctcatctag ttct

34

<210> 56

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the light  
chain of humanized anti-Fas antibody

<400> 56

gagagggtgg ccctctcccc tggagacaga gacaaagtac ctgg

44

<210> 57

<211> 44

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the light  
chain of humanized anti-Fas antibody

<400> 57  
ccaggtactt tgtctctgtc tccaggggag agggccaccc tctc 44

<210> 58  
<211> 44  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of humanized anti-Fas antibody

<400> 58  
gattcgagat tggatgcagc atagatgagg agtctgggtg cctg 44

<210> 59  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of humanized anti-Fas antibody

<400> 59  
gctgcatcca atctcgaatc tgggatccca gacaggttta gtggc 45

<210> 60  
<211> 52  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of humanized anti-Fas antibody

<400> 60  
aaaatccgcc ggctccagac gagagatggt gaggggtgaag tctgtccca ac 52

<210> 61  
<211> 58  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light

chain of humanized anti-Fas antibody

<400> 61  
ctcggtctggat gcccggat tttgcagtct attactgtca gcaaagtaat gaggatcc 58

<210> 62  
<211> 55  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the light  
chain of humanized anti-Fas antibody

<400> 62  
tgaagacaga tggcgcagcc acagtccgtt tgatttccag cctgggtgcct tgacc 55

<210> 63  
<211> 55  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the light  
chain of humanized anti-Fas antibody

<400> 63  
ggtcaggcca ccaggctgga aatcaaacgg actgtggctg caccatctgt cttca 55

<210> 64  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the light  
chain of humanized anti-Fas antibody

<400> 64  
cccgaaattct tactaacact ctccccctgtt gaagctcttt gtgac 45

<210> 65  
<211> 55  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of humanized anti-Fas antibody

<400> 65

tctgtcccag acccactgcc actaaacctg tctggatcc cagattcgag attgg

55

<210> 66

<211> 55

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of humanized anti-Fas antibody

<400> 66

gtttagtggc agtgggtctg ggacagactt cacctctacc atccatcctg tggag

55

<210> 67

<211> 55

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of humanized anti-Fas antibody

<400> 67

atggcgcaggc cacagtccgt ttgatttcca gcctggtgcc ttgaccgaac gtccg

55

<210> 68

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequencing primer for DNAs encoding the light chains of humanized anti-Fas antibodies

<400> 68

cccaagctta agaagcatcc

20

<210> 69

<211> 20

<212> DNA

<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing  
primer for DNAs encoding the light chains of  
humanized anti-Fas antibodies

<400> 69  
atctatgctg catccaatct 20

<210> 70  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing  
primer for DNAs encoding the light chains of  
humanized anti-Fas antibodies

<400> 70  
gttgttgcc tgctgaataa 20

<210> 71  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing  
primer for DNAs encoding the light chains of  
humanized anti-Fas antibodies

<400> 71  
cccgaaattct tactaacact 20

<210> 72  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing  
primer for DNAs encoding the light chains of  
humanized anti-Fas antibodies

<400> 72  
ttattcagca ggcacacaac 20

<210> 73  
<211> 20  
<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequencing primer for DNAs encoding the light chains of humanized anti-Fas antibodies

<400> 73 agattggatg cagcatagat 20

<210> 74

<211> 457

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: DNA encoding the partial peptide of the heavy chain of a humanized anti-Fas antibody

<220>

<221> CDS

<222> (21)..(455)

<220>

<221> mat peptide

<222> (78)..(455)

<220>

<221> sig peptide

<222> (21)..(77)

<400> 74  
aagcttggct tgacacctcacc atg gga tgg agc tgt atc atc ctc ttc ttg gta 53  
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val  
-15 -10

gca aca gct aca ggt gtc cac tct cag gtc caa ctg gtg cag tct ggg 101  
Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu Val Gln Ser Gly  
-5 -1 1 5

gct gag gtc aag aag cct ggg gct tca gtg aag gtg tcc tgc aag gct 149  
Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala  
10 15 20

tct ggc tac acc ttc acc agc tac tgg atg cag tgg gta aaa cag gcc 197  
Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Ala  
25 30 35 40

cct gga cag agg ctt gag tgg atg gga gag att gat cct tct gat agc 245  
Pro Gly Gln Arg Leu Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser  
45 50 55

tat act aac tac aat caa aag ttc aag ggc aag gcc aca ttg act gta	293		
Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val			
60	65	70	
gac aca tcc gct agc aca gcc tac atg gag ctc agc agc ctg aga tct	341		
Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser			
75	80	85	
gag gac acg gcg gtc tat tac tgt gca aga aat agg gac tat agt aac	389		
Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn			
90	95	100	
aac tgg tac ttc gat gtc tgg ggc gaa ggg acc ctg gtc acc gtc tcc	437		
Asn Trp Tyr Phe Asp Val Trp Gly Glu Gly Thr Leu Val Thr Val Ser			
105	110	115	120
tca gcc tcc acc aag ggc cc	457		
Ser Ala Ser Thr Lys Gly			
125			

<210> 75

<211> 145

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed partial peptide of the heavy chain of humanized anti-human Fas antibody

<400> 75

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly

-15

-10

-5

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys

-1 1

5

10

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe

15

20

25

Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Ala Pro Gly Gln Arg Leu

30

35

40

45

Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn

50

55

60

Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ala Ser

65

70

75

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val

80

85

90

Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp  
95 100 105

Val Trp Gly Glu Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
110 115 120 125

Gly

<210> 76

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding variable region in the heavy chain of a humanized anti-Fas antibody

<400> 76

gggaagcttg gcttgacctc accatggat ggagctgtat

40

<210> 77

<211> 48

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding variable region in the heavy chain of a humanized anti-Fas antibody

<400> 77

tgaagcccca ggcttcttga cctcagcccc agactgcacc agttggac

48

<210> 78

<211> 36

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding variable region in the heavy chain of a humanized anti-Fas antibody

<400> 78

tccactcaag cctctgtcca ggggcctgtt ttaccc

36

<210> 79  
<211> 52  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding variable region in the heavy chain of a humanized anti-Fas antibody

<400> 79  
gtctggggct gaggtcaaga agcctggggc ttcagtgaag gtgtcctgca ag

52

<210> 80  
<211> 39  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding variable region in the heavy chain of a humanized anti-Fas antibody

<400> 80  
caggcccctg gacagaggct tgagtggatg ggagagatt

39

<210> 81  
<211> 50  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding variable region in the heavy chain of a humanized anti-Fas antibody

<400> 81  
tcagatctca ggctgcttag ctccatgtag gctgtgctag cggatgtgtc

50

<210> 82  
<211> 44  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding variable region in the heavy chain of a humanized anti-Fas antibody

<400> 82  
tggagcttag cagcctgaga tctgaggaca cggcggtcta ttac 44

<210> 83

<211> 55

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding variable region in the heavy chain of a humanized anti-Fas antibody

<400> 83  
gatgggcct tggtggaggc tgaggagacg gtgaccaggg tcccttcgcc ccagt 55

<210> 84

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding the constant region of human immunoglobulin G1 heavy chain

<400> 84  
ggaaagcttc cgcggtcaca tggcaccacc tctcttgca 39

<210> 85

<211> 35

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding the constant region of human immunoglobulin G1 heavy chain

<400> 85  
gctctgcaga gagaagattg ggagttactg gaatc 35

<210> 86  
<211> 35  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding the constant region of human immunoglobulin G1 heavy chain

<400> 86  
tctctgcaga gcccaaattct tgtgacaaaa ctcac 35

<210> 87  
<211> 39  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of the DNA encoding the constant region of human immunoglobulin G1 heavy chain

<400> 87  
ggggaaattcg ggagcggggc ttgccggccg tcgcactca 39

<210> 88  
<211> 2077  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Designed DNA encoding the heavy chain of a humanized anti-Fas antibody

<220>  
<221> sig peptide  
<222> (27)..(83)

<220>  
<221> intron  
<222> (741)..(1131)

<220>  
<221> intron  
<222> (1177)..(1294)

<220>  
<221> intron

<222> (1625) .. (1721)

<220>

<221> exon

<222> (27) .. (740)

<220>

<221> exon

<222> (1132) .. (1176)

<220>

<221> exon

<222> (1295) .. (1624)

<220>

<221> exon

<222> (1722) .. (2042)

<220>

<221> mat peptide

<222> (84) .. (740)

<220>

<221> mat peptide

<222> (1132) .. (1176)

<220>

<221> mat peptide

<222> (1295) .. (1624)

<220>

<221> mat peptide

<222> (1722) .. (2042)

<220>

<221> CDS

<222> (27) .. (740)

<220>

<221> CDS

<222> (1132) .. (1176)

<220>

<221> CDS

<222> (1295) .. (1624)

<220>

<221> CDS

<222> (1722) .. (2042)

<400> 88

gggcgaaagc ttggcttgac ctcacc atg gga tgg agc tgt atc atc ctc ttc 53  
Met Gly Trp Ser Cys Ile Ile Leu Phe

ttg gta gca aca gct aca ggt gtc cac tct cag gtc caa ctg gtg cag	101			
Leu Val Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu Val Gln				
-10	-5	-1	1	5
tct ggg gct gag gtc aag aag cct ggg gct tca gtg aag gtg tcc tgc	149			
Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys				
10	15	20		
aag gct tct ggc tac acc ttc acc agc tac tgg atg cag tgg gta aaa	197			
Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Met Gln Trp Val Lys				
25	30	35		
cag gcc cct gga cag agg ctt gag tgg atg gga gag att gat cct tct	245			
Gln Ala Pro Gly Gln Arg Leu Glu Trp Met Gly Glu Ile Asp Pro Ser				
40	45	50		
gat agc tat act aac tac aat caa aag ttc aag ggc aag gcc aca ttg	293			
Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu				
55	60	65	70	
act gta gac aca tcc gct agc aca gcc tac atg gag ctc agc agc ctg	341			
Thr Val Asp Thr Ser Ala Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu				
75	80	85		
aga tct gag gac acg gcg gtc tat tac tgt gca aga aat agg gac tat	389			
Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr				
90	95	100		
agt aac aac tgg tac ttc gat gtc tgg ggc gaa ggg acc ctg gtc acc	437			
Ser Asn Asn Trp Tyr Phe Asp Val Trp Gly Glu Gly Thr Leu Val Thr				
105	110	115		
gtc tcc tca gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc	485			
Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro				
120	125	130		
tcc tcc aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc	533			
Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val				
135	140	145	150	
aag gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc	581			
Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala				
155	160	165		
ctg acc agc ggc gtg cac acc ttc ccg gct gtc cta cag tcc tca gga	629			
Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly				
170	175	180		
ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc agc agc ttg ggc	677			
Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Leu Gly				
185	190	195		
acc cag acc tac atc tgc aac gtg aat cac aag ccc agc aac acc aag	725			

Thr	Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys		
200					205						210						
gtg	gac	aag	aga	gtt	ggtgagagggc	cagcacaggg	agggagggtg	tctgctggaa								780	
Val	Asp	Lys	Arg	Val													
215																	
gccaggctca	gcgcgtcctgc	ctggacgcat	cccggtatg	cagtcccagt	ccagggcagc											840	
aaggcaggcc	ccgtctgcct	cttcacccgg	aggcctctgc	ccgccccact	catgctcagg											900	
gagagggtct	tctggctttt	tccccaggct	ctgggcaggc	acaggctagg	tgcccctaac											960	
ccaggccctg	cacacaaagg	ggcaggtgct	gggctcagac	ctgccaagag	ccatatccgg											1020	
gaggaccctg	cccctgacact	aagcccaccc	caaaggccaa	actctccact	ccctcagctc											1080	
ggacaccttc	tctcctccca	attccagta	actcccaatc	ttctctctgc	a	gag	ccc									1137	
						Glu	Pro										
						220											
aaa	tct	tgt	gac	aaa	act	cac	aca	tgc	cca	ccg	tgc	cca	ggtaagccag			1186	
Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Pro	Cys	Pro				
225						230											
cccaggcctc	gcctccagc	tcaaggcggg	acaggtgccc	tagtagtagcc	tgcattccagg											1246	
gacaggcccc	agccgggtgc	tgacacgtcc	acctccatct	cttcctca	gca	cct	gaa									1303	
						Ala	Pro	Glu									
						235											
ctc	ctg	ggg	gga	ccg	tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	ccc	aag	gac		1351
Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp		
240						245						250					
acc	ctc	atg	atc	tcc	cg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	gac		1399
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp		
255						260						265					
gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	ggc		1447
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly		
270						275						280					285
gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	ccg	ccg	gag	gag	cag	tac	aac		1495
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn		
290						295										300	
agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	cag	gac	tgg		1543
Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp		
305						310						315					
ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	gtc	tcc	aac	aaa	gcc	ctc	cca		1591
Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro		
320						325						330					

gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggtgggaccc gtggggtgcg 1644  
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys  
     335                         340

agggccacat ggacagaggc cggctcgcc caccctctgc cctgagagtg accgctgtac 1704  
 caaacctctgt ccctaca ggg cag ccc cga gaa cca cag gtg tac acc ctg   1754  
                   Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu  
                   345                         350                         355

ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg acc tgc   1802  
 Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys  
     360                         365                         370

ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc   1850  
 Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser  
     375                         380                         385

aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac   1898  
 Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp  
     390                         395                         400

tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc   1946  
 Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser  
     405                         410                         415

agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct   1994  
 Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala  
     420                         425                         430                         435

ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa   2042  
 Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys  
     440                         445                         450

tgagtgcgac ggccggcaag ccccgctccc gaatt                                 2077

<210> 89  
 <211> 470  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Designed heavy  
       chain of humanized anti-Fas antibody

<400> 89  
 Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
     -15                         -10                         -5

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
     -1      1                         5                         10

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
15 20 25

Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Ala Pro Gly Gln Arg Leu  
30 35 40 45

Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn  
50 55 60

Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Ala Ser  
65 70 75

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val  
80 85 90

Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp  
95 100 105

Val Trp Gly Glu Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
110 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
145 150 155

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
160 165 170

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
175 180 185

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
190 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro  
210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
225 230 235

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
240 245 250

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
255 260 265

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
270 275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
305 310 315

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
320 325 330

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
335 340 345

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
350 355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
400 405 410

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
415 420 425

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
430 435 440 445

Ser Leu Ser Pro Gly Lys  
450

<210> 90

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 90

acagccggga aggtgtgcac

20

<210> 91

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 91  
agacaccctc cctccctgtg

20

<210> 92  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 92  
gtgcaggggcc tgggttaggg

20

<210> 93  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 93  
gcacgggtggg catgtgtgag

20

<210> 94  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 94  
gttttgggggg gaagaggaag

20

<210> 95  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a

humanized anti-Fas antibody

<400> 95  
ccagtcctgg tgcaggacgg

20

<210> 96  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 96  
cctgtggttc tcggggctgc

20

<210> 97  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 97  
cgtggtcttg tagttgttct

20

<210> 98  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 98  
cttcctcttc ccccaaaaac

20

<210> 99  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 99

ccgtcctgca ccaggactgg

20

<210> 100

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 100

gcagccccga gaaccacagg

20

<210> 101

<211> 20

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 101

agaacaacta caagaccacg

20

<210> 102

<211> 19

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 102

gcctgacatc tgaggactc

19

<210> 103

<211> 19

<212> DNA

<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing  
primer for a DNA encoding the heavy chain of a  
humanized anti-Fas antibody

<400> 103  
gagtcctcag atgtcaggc

19

<210> 104  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing  
primer for a DNA encoding the heavy chain of a  
humanized anti-Fas antibody

<400> 104  
gagcagtaact cgttgctgcc gcgcgcgcgc ccag

34

<210> 105  
<211> 24  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing  
primer for a DNA encoding the heavy chain of a  
humanized anti-Fas antibody

<400> 105  
ggtatggctg attaatgatc aatg

24

<210> 106  
<211> 768  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Designed DNA  
encoding the light chain of a humanized anti-Fas  
antibody

<220>  
<221> CDS  
<222> (40)..(753)

<220>  
<221> mat peptide  
<222> (100)..(753)

<220>  
 <221> sig peptide  
 <222> (40)..(99)

<400> 106

cccaagctta	agaagcatcc	tctcatctag	ttctcagag	atg gag aca gac aca	54											
				Met Glu Thr Asp Thr												
				-20												
atc	ctg	cta	tgg	gtg	ctg	ctc	tgg	gtt	cca	ggc	tcc	act	ggt	gag	102	
Ile	Leu	Leu	Trp	Val	Leu	Leu	Trp	Val	Pro	Gly	Ser	Thr	Gly	Glu		
-15					-10				-5			-1		1		
att	gtg	ctc	acc	caa	tct	cca	ggt	act	ttg	tct	ctg	tct	cca	ggg	gag	150
Ile	Val	Leu	Thr	Gln	Ser	Pro	Gly	Thr	Leu	Ser	Leu	Ser	Pro	Gly	Glu	
					5				10				15			
agg	gcc	acc	ctc	tcc	tgc	aag	gcc	agc	caa	agt	gtt	gat	tat	gat	ggt	198
Arg	Ala	Thr	Leu	Ser	Cys	Lys	Ala	Ser	Gln	Ser	Val	Asp	Tyr	Asp	Gly	
					20				25			30				
gat	agt	tat	atg	aac	tgg	tac	caa	cag	aaa	cca	gga	cag	gca	ccc	aga	246
Asp	Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Gln	Ala	Pro	Arg	
					35				40			45				
ctc	ctc	atc	tat	gct	gca	tcc	aat	ctc	gaa	tct	ggg	atc	cca	gac	agg	294
Leu	Leu	Ile	Tyr	Ala	Ala	Ser	Asn	Leu	Glu	Ser	Gly	Ile	Pro	Asp	Arg	
					50				55			60		65		
ttt	agt	ggc	agt	ggg	tct	ggg	aca	gac	ttc	acc	ctc	acc	atc	tct	cgt	342
Phe	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Arg	
					70				75				80			
ctg	gag	ccg	gag	gat	ttt	gca	gtc	tat	tac	tgt	cag	caa	agt	aat	gag	390
Leu	Glu	Pro	Glu	Asp	Phe	Ala	Val	Tyr	Tyr	Cys	Gln	Gln	Ser	Asn	Glu	
					85				90			95				
gat	cct	cg	ac	tt	gg	ca	gg	ac	a	ct	gaa	atc	aa	cg	act	438
Asp	Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Leu	Glu	Ile	Lys	Arg	Thr	
					100				105			110				
gtg	gct	gca	cca	tct	gtc	ttc	atc	ttc	ccg	cca	tct	gat	gag	cag	ttg	486
Val	Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	
					115				120			125				
aaa	tct	gga	act	gg	tct	gtt	gtg	tgc	ctg	ctg	aat	aac	ttc	tat	ccc	534
Lys	Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	
					130				135			140		145		
aga	gag	gg	aaa	gta	cag	tgg	aaa	gtg	gat	aac	gg	ctc	caa	tcg	ggt	582
Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	
					150				155			160				
aac	tcc	cag	gag	agt	gtc	aca	gag	cag	gac	agc	aag	gac	agc	acc	tac	630

Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr			
165	170	175	
agc ctc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac			678
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His			
180	185	190	
aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc			726
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val			
195	200	205	
aca aag agc ttc aac agg gga gag tgt tagtaagaat tcggg			768
Thr Lys Ser Phe Asn Arg Gly Glu Cys			
210	215		

<210> 107

<211> 238

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed light chain of humanized anti-Fas antibody

<400> 107

Met Glu Thr Asp Thr Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro			
-20	-15	-10	-5

Gly Ser Thr Gly Glu Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser			
-1    1	5	10	

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser			
15	20	25	

Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro			
30	35	40	

Gly Gln Ala Pro Arg Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser			
45	50	55	60

Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr			
65	70	75	

Leu Thr Ile Ser Arg Leu Glu Pro Glu Asp Phe Ala Val Tyr Tyr Cys			
80	85	90	

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Lys Leu			
95	100	105	

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro			
110	115	120	

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
125 130 135 140

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn  
145 150 155

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
160 165 170

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
175 180 185

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
190 195 200

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
205 210 215

<210> 108

<211> 768

<212> DNA

<213> Artificial Sequence

<220>

<221> CDS

<222> (40)..(753)

<220>

<221> mat peptide

<222> (100)..(753)

<220>

<221> sig peptide

<222> (40)..(99)

<220>

<223> Description of Artificial Sequence: Designed DNA  
encoding the light chain of a humanized anti-Fas  
antibody

<400> 108

cccaagctta agaaggcatcc tctcatcttag ttctcagag atg gag aca gac aca 54  
Met Glu Thr Asp Thr  
-20

atc ctg cta tgg gtg ctg ctc tgg gtt cca ggc tcc act ggt gag 102  
Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro Gly Ser Thr Gly Glu  
-15 -10 -5 -1 1

att gtg ctc acc caa tct cca ggt act ttg tct ctg tct cca ggg gag 150  
Ile Val Leu Thr Gln Ser Pro Gly Thr Leu Ser Leu Ser Pro Gly Glu  
5 10 15

agg gcc acc ctc tcc tgc aag gcc agc caa agt gtt gat tat gat ggt	198
Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp Gly	
20 25 30	
gat agt tat atg aac tgg tac caa cag aaa cca gga cag gca ccc aga	246
Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Arg	
35 40 45	
ctc ctc atc tat gct gca tcc aat ctc gaa tct ggg atc cca gac agg	294
Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Ile Pro Asp Arg	
50 55 60 65	
ttt agt ggc agt ggg tct ggg aca gac ttc acc ctc acc atc cat cct	342
Phe Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile His Pro	
70 75 80	
gtg gag gag gag gat gct gca acc tat tac tgt cag caa agt aat gag	390
Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu	
85 90 95	
gat cct cg <sup>g</sup> acg ttc ggt caa ggc acc aag ctg gaa atc aaa cg <sup>g</sup> act	438
Asp Pro Arg Thr Phe Gly Gln Gly Thr Lys Leu Glu Ile Lys Arg Thr	
100 105 110	
gtg gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag ttg	486
Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu	
115 120 125	
aaa tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc	534
Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro	
130 135 140 145	
aga gag gcc aaa gta cag tgg aaa gtg gat aac gcc ctc caa tcg ggt	582
Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly	
150 155 160	
aac tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc tac	630
Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr	
165 170 175	
agc ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac	678
Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His	
180 185 190	
aaa gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc	726
Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val	
195 200 205	
aca aag agc ttc aac agg gga gag tgt tagtaagaat tcggg	768
Thr Lys Ser Phe Asn Arg Gly Glu Cys	
210 215	

<211> 238

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed light chain of humanized anti-Fas antibody

<400> 109

Met	Glu	Thr	Asp	Thr	Ile	Leu	Leu	Trp	Val	Leu	Leu	Leu	Trp	Val	Pro
-20					-15					-10					-5

Leu Ser Pro Gly Glu Arg Ala Thr Leu Ser Cys Lys Ala Ser Gln Ser  
15 20 25

Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro  
30 35 40

Gly Gln Ala Pro Arg Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
45 50 55 60

Gly Ile Pro Asp Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
 65 70 75

Leu Thr Ile His Pro Val Glu Glu Glu Asp Ala Ala Thr Tyr Tyr Cys  
80 85 90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Lys Leu  
95 100 105

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro  
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
125 130 135 140

Asn	Asn	Phe	Tyr	Pro	Arg	Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn
				145					150				155		

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
160 165 170

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
175 180 185

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
190 195 200

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
205 210 215

<210> 110  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the light chain of a humanized anti-Fas antibody

<400> 110  
ggtgagattg tgctcaccca atctccagg 29

<210> 111  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the light chain of a humanized anti-Fas antibody

<400> 111  
cctggagatt gggtgagcac aatctcacc 29

<210> 112  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the light chain of a humanized anti-Fas antibody

<400> 112  
ccatctctcg tctggagccg gaggatttg c 31

<210> 113  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the light chain of a humanized anti-Fas antibody

<400> 113  
gcaaaaatcct ccggctccag acgagagatg g 31

<210> 114  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the light chain of a humanized anti-Fas antibody

<400> 114  
caaggcacca agctggaaat caaacggact g 31

<210> 115  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the light chain of a humanized anti-Fas antibody

<400> 115  
cagtccgtt gatttccagc ttgggtgcctt g 31

<210> 116  
<211> 2071  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Designed DNA encoding the heavy chain of a humanized anti-Fas antibody

<220>  
<221> sig peptide  
<222> (21)..(77)

<220>  
<221> intron  
<222> (735)..(1125)

<220>  
<221> intron  
<222> (1171)..(1288)

<220>  
<221> intron  
<222> (1619)..(1715)

<220>  
<221> exon  
<222> (21)..(734)

<220>  
<221> exon  
<222> (1126)..(1170)

<220>  
<221> exon  
<222> (1289)..(1618)

<220>  
<221> exon  
<222> (1716)..(2036)

<220>  
<221> mat peptide  
<222> (78)..(734)

<220>  
<221> mat peptide  
<222> (1126)..(1170)

<220>  
<221> mat peptide  
<222> (1289)..(1618)

<220>  
<221> mat peptide  
<222> (1716)..(2036)

<220>  
<221> CDS  
<222> (21)..(734)

<220>  
<221> CDS  
<222> (1126)..(1170)

<220>  
<221> CDS  
<222> (1289)..(1618)

<220>  
<221> CDS  
<222> (1716)..(2036)

<400> 116  
aagcttggct tgacacctacc atg gga tgg agc tgt atc atc ctc ttc ttg gta 53  
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val  
-15 -10

gca aca gct aca ggt gtc cac tct cag gtc caa ctg gtg cag tct ggg 101



aag aga gtt ggtgagaggg cagcacaggg agggagggtg tctgctggaa 774  
 Lys Arg Val  
  
 gccaggctca gcgctcctgc ctggacgcat cccggctatg cagtcccagt ccagggcagc 834  
 aaggcaggcc ccgtctgcct cttcaccgg aggccctctgc cggccccact catgctcagg 894  
 gagagggtct tctggcttt tccccaggct ctgggcaggc acaggctagg tgcccctaac 954  
 ccaggccctg cacacaaagg ggcaggtgct gggctcagac ctgccaagag ccatatccgg 1014  
 gaggaccctg cccctgacct aagcccaccc caaaggccaa actctccact ccctcagctc 1074  
 ggacaccccttc ttcctccca gattccagta actcccaatc ttctctctgc a gag ccc 1131  
Glu Pro  
220  
  
 aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca ggtaagccag 1180  
 Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro  
225230  
  
 cccaggcctc gccctccagc tcaaggcggg acaggtgccc tagagtagcc tgcataccagg 1240  
 gacaggcccc agccgggtgc tgacacgtcc acctccatct cttcctca gca cct gaa 1297  
Ala Pro Glu  
235  
  
 ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac 1345  
 Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
240245250  
  
 acc ctc atg atc tcc ccg acc cct gag gtc aca tgc gtg gtg gtg gac 1393  
 Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
255260265  
  
 gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc 1441  
 Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
270275280285  
  
 gtg gag gtg cat aat gcc aag aca aag ccg ccg gag gag cag tac aac 1489  
 Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
290295300  
  
 agc acg tac cgt gtg gtc agc gtc ctc acc gtc ctg cac cag gac tgg 1537  
 Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
305310315  
  
 ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca 1585  
 Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
320325330  
  
 gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggtgggaccc gtggggtgcg 1638  
 Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys  
335340

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<210> 117  
<211> 470  
<212> PRT  
<213> Artificial Sequence
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<220>  
<223> Description of Artificial Sequence: Designed heavy  
chain of humanized anti-Fas antibody

<400> 117  
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
-15 -10 -5

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
 -1 1 5 10

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
15 20 25

Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Ala Pro Gly Gln Gly Leu  
30 35 40 45

Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn  
50 55 60

Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Thr Ser  
65 70 75

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val  
80 85 90

Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp  
95 100 105

Val Trp Gly Glu Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
110 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
145 150 155

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
160 165 170

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
175 180 185

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
190 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro  
210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
225 230 235

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
240 245 250

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
255 260 265

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
270 275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
305 310 315

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
320 325 330

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
335 340 345

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
350 355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
400 405 410

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
415 420 425

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
430 435 440 445

Ser Leu Ser Pro Gly Lys  
450

<210> 118

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of a DNA encoding the heavy  
chain of a humanized anti-Fas antibody

<400> 118

caggcccctg gacagggcct tgagtggatg

30

<210> 119

<211> 30

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of a DNA encoding the heavy  
chain of a humanized anti-Fas antibody

<400> 119

catccactca aggccctgtc caggggcctg

30

<210> 120  
<211> 39  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of a DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 120  
gctgagctcc atgtaggctg tgcttagtgaa tgtgtctac 39

<210> 121  
<211> 33  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a DNA fragment including SR alpha promoter

<400> 121  
tgcacgcgtg gctgtggaat gtgtgtcagt tag 33

<210> 122  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a DNA fragment including SR alpha promoter

<400> 122  
tccgaagctt ttagagcaga agtaaacactt c 31

<210> 123  
<211> 36  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a DNA fragment including SR alpha promoter

<400> 123  
aaagcggccg ctgcttagctt ggctgtggaa tgtgtg 36

<210> 124  
<211> 34  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a DNA encoding the kappa light chain of human immunoglobulin

<400> 124  
aagcttatgg acatgagggt ccccgctctg ctcc

34

<210> 125  
<211> 729  
<212> DNA  
<213> Homo sapiens

<400> 125  
aagcttatgg acatgagggt ccccgctctg ctcctgggc tcctgctact ctggctccga 60  
gggccagat gtgacatcca gatgaccagg tctccatcct ccctgtctgc atctgttagga 120  
gacagagtca ccatcacttg ccggcaagt cagagcatta gcagctattt aaattggat 180  
cagcagaaac cagggaaagc ccctaagctc ctgatctatg ctgcattccag tttgcaaagt 240  
gggtccccat caaggttcag tggcagtgga tctggacag atttcactct caccatcagc 300  
agtctgcaac ctgaagattt tgcaacttac tactgtcaac agagttacag taccctcga 360  
acgttcggcc aaggaccaa ggtggaaatc aaacgaactg tggctgcacc atctgtttc 420  
atcttcccgc catctgatga gcagttgaaa tctggaaactg cctctgttgt gtgcctgctg 480  
aataacttct atcccagaga gcccaaagta cagtggagg tggataacgc cctccaatcg 540  
ggtaactccc aggagagtgt tacagagcag gacagcaagg acagcaccta cagcctcagc 600  
agcacccctga cgctgagcaa agcagactac gagaaacaca aagtctacgc ctgcgaagtc 660  
acccatcagg gcctgagctc gcccgtcaca aagagttca acaggggaga gtgttagtaa 720  
gaattcggg 729

<210> 126  
<211> 767  
<212> DNA  
<213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Designed DNA  
 encoding the light chain of a humanized anti-Fas  
 antibody

<220>  
 <221> CDS  
 <222> (39)..(752)

<220>  
 <221> mat peptide  
 <222> (99)..(752)

<220>  
 <221> sig peptide  
 <222> (39)..(98)

<400> 126

ccaagcttaa	gaagcatcct	ctcatctagt	tctcagag	atg	gag	aca	gac	aca	atc	56						
				Met	Glu	Thr	Asp	Thr	Ile							
				-20						-15						
ctg	cta	tgg	gtg	ctg	ctc	tgg	gtt	cca	ggc	tcc	act	ggt	gac	att	104	
Leu	Leu	Trp	Val	Leu	Leu	Leu	Trp	Val	Pro	Gly	Ser	Thr	Gly	Asp	Ile	
-10									-5			-1		1		
gtg	ctc	acc	caa	tct	cca	tcc	ctg	tct	gca	tct	gta	gga	gac	aga	152	
Val	Leu	Thr	Gln	Ser	Pro	Ser	Ser	Leu	Ser	Ala	Ser	Val	Gly	Asp	Arg	
5									10			15				
gtc	acc	atc	act	tgc	aag	gcc	agc	caa	agt	gtt	gat	tat	gat	ggt	200	
Val	Thr	Ile	Thr	Cys	Lys	Ala	Ser	Gln	Ser	Val	Asp	Tyr	Asp	Gly	Asp	
20									25			30				
agt	tat	atg	aac	tgg	tac	caa	cag	aaa	cca	gga	aag	gca	ccc	aag	248	
Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Ile	
35									40			45		50		
ctc	atc	tat	gct	gca	tcc	aat	ttg	gaa	agt	ggg	gtc	cca	tca	agg	296	
Leu	Ile	Tyr	Ala	Ala	Ser	Asn	Leu	Glu	Ser	Gly	Val	Pro	Ser	Arg	Phe	
55									60			65				
agt	gga	agt	gga	tct	ggg	aca	gat	ttt	act	ctc	acc	atc	agc	agc	344	
Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Ile	
70									75			80				
cag	cct	gaa	gat	ttt	gca	acc	tac	tac	tgt	cag	caa	agt	aac	gag	gat	392
Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Asn	Glu	Asp	
85									90			95				
cct	cgg	acg	ttc	ggc	caa	ggc	acc	aag	gtg	gaa	atc	aaa	cgg	act	gtg	440
Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Glu	Ile	Lys	Arg	Thr	Val	
100									105			110				



Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
65 70 75

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys  
80 85 90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Lys Val  
95 100 105

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro  
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
125 130 135 140

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn  
145 150 155

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
160 165 170

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
175 180 185

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
190 195 200

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
205 210 215

<210> 128

<211> 767

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed DNA  
encoding the light chain of a humanized anti-Fas  
antibody

<220>

<221> CDS

<222> (39)..(752)

<220>

<221> mat peptide

<222> (99)..(752)

<220>

<221> sig peptide

<222> (39)..(98)

<400> 128

ccaagcttaa gaagcatcct ctcatctagt tctcagag atg gag aca gac aca atc	56
Met Glu Thr Asp Thr Ile	
-20	-15
ctg cta tgg gtg ctg ctc tgg gtt cca ggc tcc act ggt gac att	104
Leu Leu Trp Val Leu Leu Trp Val Pro Gly Ser Thr Gly Asp Ile	
-10	-5
-1	1
gtg ctc acc caa tct cca tcc tcc ctg tct gca tct gta gga gac aga	152
Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg	
5	10
15	
gtc acc atc act tgc aag gcc agc caa agt gtt gat tat gat ggt gat	200
Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp Gly Asp	
20	25
30	
agt tat atg aac tgg tac caa cag aaa cca gga cag gca ccc aag ctc	248
Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro Gly Gln Ala Pro Lys Leu	
35	40
45	50
ctc atc tat gct gca tcc aat ttg gaa agt ggg gtc cca tca agg ttc	296
Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser Gly Val Pro Ser Arg Phe	
55	60
65	
agt gga agt gga tct ggg aca gat ttt act ctc acc atc agc agc ctg	344
Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Ser Leu	
70	75
80	
cag cct gaa gat ttt gca acc tac tac tgt caa cag agt aac gag gat	392
Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys Gln Gln Ser Asn Glu Asp	
85	90
95	
cct cga acg ttc ggc caa ggc acc aag gtg gaa atc aaa cggt act gtg	440
Pro Arg Thr Phe Gly Gln Gly Thr Lys Val Glu Ile Lys Arg Thr Val	
100	105
110	
gct gca cca tct gtc ttc atc ttc ccg cca tct gat gag cag ttg aaa	488
Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln Leu Lys	
115	120
125	130
tct gga act gcc tct gtt gtg tgc ctg ctg aat aac ttc tat ccc aga	536
Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr Pro Arg	
135	140
145	
gag gcc aaa gta cag tgg aaa gtg gat aac gcc ctc caa tcg ggt aac	584
Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser Gly Asn	
150	155
160	
tcc cag gag agt gtc aca gag cag gac agc aag gac agc acc tac agc	632
Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr Tyr Ser	
165	170
175	
ctc agc agc acc ctg acg ctg agc aaa gca gac tac gag aaa cac aaa	680

Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys His Lys  
 180 185 190

gtc tac gcc tgc gaa gtc acc cat cag ggc ctg agc tcg ccc gtc aca 728  
 Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro Val Thr  
 195 200 205 210

aag agc ttc aac agg gga gag tgt tagtaagaat tcggg 767  
 Lys Ser Phe Asn Arg Gly Glu Cys  
 215

<210> 129  
 <211> 238  
 <212> PRT  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: Designed light chain of humanized anti-Fas antibody

<400> 129  
 Met Glu Thr Asp Thr Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro -20 -15 -10 -5

Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser -1 1 5 10

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser 15 20 25

Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro 30 35 40

Gly Gln Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser 45 50 55 60

Gly Val Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr 65 70 75

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys 80 85 90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Lys Val 95 100 105

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro 110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu 125 130 135 140

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn 145 150 155

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
160 165 170

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
175 180 185

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
190 195 200

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
205 210 215

<210> 130

<211> 778

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed DNA  
encoding the light chain of a humanized anti-Fas  
antibody

<220>

<221> CDS

<222> (39)..(752)

<220>

<221> mat peptide

<222> (99)..(752)

<220>

<221> sig peptide

<222> (39)..(98)

<400> 130

ccaagcttaa gaaggatcct ctcatctagt tctcagag atg gag aca gac aca atc 56  
Met Glu Thr Asp Thr Ile  
-20 -15

ctg cta tgg gtg ctg ctc tgg gtt cca ggc tcc act ggt gac att 104  
Leu Leu Trp Val Leu Leu Trp Val Pro Gly Ser Thr Gly Asp Ile  
-10 -5 -1 1

gtg ctc acc caa tct cca tcc tcc ctg tct gca tct gta gga gac aga 152  
Val Leu Thr Gln Ser Pro Ser Ser Leu Ser Ala Ser Val Gly Asp Arg  
5 10 15

gtc acc atc act tgc aag gcc agc caa agt gtt gat tat gat ggt gat 200  
Val Thr Ile Thr Cys Lys Ala Ser Gln Ser Val Asp Tyr Asp Gly Asp  
20 25 30

agt tat atg aac tgg tac caa cag aaa cca gga aag gca ccc aaa ctc 248

Ser	Tyr	Met	Asn	Trp	Tyr	Gln	Gln	Lys	Pro	Gly	Lys	Ala	Pro	Lys	Leu		
35				40					45						50		
ctc	atc	tac	gct	gca	tcc	aat	ttg	gaa	tca	ggg	atc	cca	tca	agg	ttc	296	
Leu	Ile	Tyr	Ala	Ala	Ser	Asn	Leu	Glu	Ser	Gly	Ile	Pro	Ser	Arg	Phe		
				55				60						65			
agt	gga	agt	gga	tct	ggg	aca	gat	ttt	act	ctc	acc	atc	agc	agc	ctg	344	
Ser	Gly	Ser	Gly	Ser	Gly	Ser	Gly	Thr	Asp	Phe	Thr	Leu	Thr	Ile	Ser	Ser	Leu
				70				75						80			
cag	cct	gag	gat	ttt	gca	acc	tat	tac	tgt	cag	caa	agt	aat	gag	gat	392	
Gln	Pro	Glu	Asp	Phe	Ala	Thr	Tyr	Tyr	Cys	Gln	Gln	Ser	Asn	Glu	Asp		
				85				90						95			
cct	cg	acg	ttc	ggt	caa	ggc	acc	aag	gtg	gaa	atc	aaa	cg	act	gtg	440	
Pro	Arg	Thr	Phe	Gly	Gln	Gly	Thr	Lys	Val	Gl	Ile	Lys	Arg	Thr	Val		
				100				105						110			
gct	gca	cca	tct	gtc	ttc	atc	ttc	ccg	cca	tct	gat	gag	cag	ttg	aaa	488	
Ala	Ala	Pro	Ser	Val	Phe	Ile	Phe	Pro	Pro	Ser	Asp	Glu	Gln	Leu	Lys		
				115				120						130			
tct	gga	act	gcc	tct	gtt	gtg	tgc	ctg	ctg	aat	aac	ttc	tat	ccc	aga	536	
Ser	Gly	Thr	Ala	Ser	Val	Val	Cys	Leu	Leu	Asn	Asn	Phe	Tyr	Pro	Arg		
				135				140						145			
gag	gcc	aaa	gta	cag	tgg	aag	gtg	gat	aac	gcc	ctc	caa	tcg	ggt	aac	584	
Glu	Ala	Lys	Val	Gln	Trp	Lys	Val	Asp	Asn	Ala	Leu	Gln	Ser	Gly	Asn		
				150				155						160			
tcc	cag	gag	agt	gtc	aca	gag	cag	gac	agc	aag	gac	agc	acc	tac	agc	632	
Ser	Gln	Glu	Ser	Val	Thr	Glu	Gln	Asp	Ser	Lys	Asp	Ser	Thr	Tyr	Ser		
				165				170						175			
ctc	agc	agc	acc	ctg	acg	ctg	agc	aaa	gca	gac	tac	gag	aaa	cac	aaa	680	
Leu	Ser	Ser	Thr	Leu	Thr	Leu	Ser	Lys	Ala	Asp	Tyr	Glu	Lys	His	Lys		
				180				185						190			
gtc	tac	gcc	tgc	gaa	gtc	acc	cat	cag	ggc	ctg	agc	tcg	ccc	gtc	aca	728	
Val	Tyr	Ala	Cys	Glu	Val	Thr	His	Gln	Gly	Leu	Ser	Ser	Pro	Val	Thr		
				195				200						205		210	
aag	agc	ttc	aac	agg	gga	gag	tgt	tagtaagaat	tcgggaagcc	gaattc						778	
Lys	Ser	Phe	Asn	Arg	Gly	Glu	Cys										
				215													

<210> 131  
<211> 238  
<212> PRT  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed light chain of humanized anti-Fas antibody

<400> 131

Met Glu Thr Asp Thr Ile Leu Leu Trp Val Leu Leu Leu Trp Val Pro  
-20 -15 -10 -5

Gly Ser Thr Gly Asp Ile Val Leu Thr Gln Ser Pro Ser Ser Leu Ser  
-1 1 5 10

Ala Ser Val Gly Asp Arg Val Thr Ile Thr Cys Lys Ala Ser Gln Ser  
15 20 25

Val Asp Tyr Asp Gly Asp Ser Tyr Met Asn Trp Tyr Gln Gln Lys Pro  
30 35 40

Gly Lys Ala Pro Lys Leu Leu Ile Tyr Ala Ala Ser Asn Leu Glu Ser  
45 50 55 60

Gly Ile Pro Ser Arg Phe Ser Gly Ser Gly Ser Gly Thr Asp Phe Thr  
65 70 75

Leu Thr Ile Ser Ser Leu Gln Pro Glu Asp Phe Ala Thr Tyr Tyr Cys  
80 85 90

Gln Gln Ser Asn Glu Asp Pro Arg Thr Phe Gly Gln Gly Thr Lys Val  
95 100 105

Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro  
110 115 120

Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu  
125 130 135 140

Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn  
145 150 155

Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser  
160 165 170

Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala  
175 180 185

Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly  
190 195 200

Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys  
205 210 215

<210> 132

<211> 41

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 132  
agggaggatg gagattgggt gagcacaatg tcaccagtgg a 41

<210> 133  
<211> 39  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 133  
atttgctca cccaatctcc atcctccctg tctgcatct 39

<210> 134  
<211> 42  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 134  
atcaacactt tggctggcct tgcaagtgat ggtgactctg tc 42

<210> 135  
<211> 40  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 135  
ccatcacttg caaggccagc caaagtgttg attatgatgg 40

<210> 136  
<211> 48  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 136  
agtttcgaga ttggatgcag catagatgag gagtttgggt gcctttcc 48

<210> 137  
<211> 45  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 137  
cccaagctcc tcatcttatgc tgcattccaaat ttggaaagtg gggtc 45

<210> 138  
<211> 44  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 138  
ttggccgaac gttcgaggat cctcgttact ctgttgacag tagt 44

<210> 139  
<211> 44  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 139

actactgtca acagagtaac gaggatcctc gaacgttcgg ccaa

44

<210> 140

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 140

ctcatctatg ctgcatccaa tttggaaagt gggatcccat caagg

45

<210> 141

<211> 45

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the light chain of a humanized anti-Fas antibody

<400> 141

attggatgca gcatacatga ggagcttggg tgcctgtcct ggttt

45

<210> 142

<211> 2073

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed DNA encoding the heavy chain of a humanized anti-Fas antibody

<220>

<221> sig peptide

<222> (23)..(79)

<220>

<221> intron

<222> (737)..(1127)

<220>

<221> intron

<222> (1173)..(1290)

<220>  
<221> intron  
<222> (1621) .. (1717)

<220>  
<221> exon  
<222> (23) .. (736)

<220>  
<221> exon  
<222> (1128) .. (1172)

<220>  
<221> exon  
<222> (1291) .. (1620)

<220>  
<221> exon  
<222> (1718) .. (2038)

<220>  
<221> mat peptide  
<222> (80) .. (736)

<220>  
<221> mat peptide  
<222> (1128) .. (1172)

<220>  
<221> mat peptide  
<222> (1291) .. (1620)

<220>  
<221> mat peptide  
<222> (1718) .. (2038)

<220>  
<221> CDS  
<222> (23) .. (736)

<220>  
<221> CDS  
<222> (1128) .. (1172)

<220>  
<221> CDS  
<222> (1291) .. (1620)

<220>  
<221> CDS  
<222> (1718) .. (2038)

ccaagcttgg cttgacctca cc atg gga tgg agc tgt atc atc ctc ttc ttg	52
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu	
-15	-10
 gta gca aca gct aca ggt gtc cat tct cag gtc caa ctg gtg cag tct	100
Val Ala Thr Ala Thr Gly Val His Ser Gln Val Gln Leu Val Gln Ser	
-5 -1 1 5	
 ggg gct gag gtc aag aag cct ggg gct tca gtg aag gtg tcc tgc aag	148
Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys Lys	
10 15 20	
 gct tct ggc tac acc ttc acc agc tac tgg atg cag tgg gta aaa cag	196
Ala Ser Gly Tyr Thr Phe Thr Ser Tyr Trp Met Gln Trp Val Lys Gln	
25 30 35	
 gcc cct gga cag gga ctt gag tgg atg gga gag att gat cct tct gat	244
Ala Pro Gly Gln Gly Leu Glu Trp Met Gly Glu Ile Asp Pro Ser Asp	
40 45 50 55	
 agc tat act aac tac aatcaa aag ttc aag ggc aag gcc aca ttg act	292
Ser Tyr Thr Asn Tyr Asn Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr	
60 65 70	
 gta gac aca tcc act agc aca gcc tac atg gag ctc agc agc ctg aga	340
Val Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg	
75 80 85	
 tct gag gac acg gcg gtc tat tac tgt gca aga aat agg gac tat agt	388
Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser	
90 95 100	
 aac aac tgg tac ttc gat gtc tgg ggc caa ggt aca ctg gtc acc gtc	436
Asn Asn Trp Tyr Phe Asp Val Trp Gly Gln Gly Thr Leu Val Thr Val	
105 110 115	
 tcc tca gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc tcc	484
Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro Ser	
120 125 130 135	
 tcc aag agc acc tct ggg ggc aca gcg gcc ctg ggc tgc ctg gtc aag	532
Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu Gly Cys Leu Val Lys	
140 145 150	
 gac tac ttc ccc gaa ccg gtg acg gtg tcg tgg aac tca ggc gcc ctg	580
Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp Asn Ser Gly Ala Leu	
155 160 165	
 acc agc ggc gtg cac acc ttc ccg gct gtc cta cag tcc tca gga ctc	628
Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly Leu	
170 175 180	
 tac tcc ctc agc agc gtg gtg acc gtg ccc tcc agc agc ttg ggc acc	676

Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	
185					190					195						
cag	acc	tac	atc	tgc	aac	gtg	aat	cac	aag	ccc	agc	aac	acc	aag	gtg	724
Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	
200					205					210					215	
gac	aag	aga	gtt	ggtgagagggc	cagcacaggg	agggaggggtg	tctgctggaa									776
Asp	Lys	Arg	Val													
gccaggctca	gcgctcctgc	ctggacgcat	cccggtatg	cagtcccagt	ccagggcagc	836										
aaggcaggcc	ccgtctgcct	cttcacccgg	aggcctctgc	ccgccccact	catgctcagg	896										
gagaggggtct	tctggcttt	tccccaggct	ctgggcagggc	acaggctagg	tgccccta	956										
ccaggccctg	cacacaaagg	ggcaggtgct	gggctcagac	ctgccaagag	ccatatccgg	1016										
gaggaccctg	cccctgaccc	aagcccaccc	caaaggccaa	actctccact	ccctcagctc	1076										
ggacaccttc	tctcctccca	gattccagta	actcccaatc	ttctctctgc	a	gag	ccc									1133
						Glu	Pro									
						220										
aaa	tct	tgt	gac	aaa	act	cac	aca	tgc	cca	ccg	tgc	cca	ggtaagccag			1182
Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Pro	Cys	Pro			
225				230												
cccaggcctc	gccctccagc	tcaaggcggg	acaggtgccc	tagatagcc	tgc	atccagg	1242									
gacaggcccc	agccgggtgc	tgacacgtcc	acctccatct	cttcctca	gca	cct	gaa									1299
						Ala	Pro	Glu								
						235										
ctc	ctg	ggg	gga	ccg	tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	ccc	aag	gac	1347
Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	
240				245								250				
acc	ctc	atg	atc	tcc	cg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	gac	1395
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	
255				260							265					
gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	ggc	1443
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	
270				275						280					285	
gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	ccg	ccg	gag	gag	cag	tac	aac	1491
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	
290				295									300			
agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	cag	gac	tgg	1539
Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	
305				310									315			

ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca	1587
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro	
320 325 330	
gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggtgggaccc gtggggtgcg	1640
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys	
335 340	
aggcccacat ggacagagggc cggctcgcc caccctctgc cctgagagtg accgctgtac	1700
caacctctgt ccctaca ggg cag ccc cga gaa cca cag gtg tac acc ctg	1750
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu	
345 350 355	
ccc cca tcc cg <sup>g</sup> gag gag atg acc aag aac cag gtc agc ctg acc tgc	1798
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys	
360 365 370	
ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc	1846
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser	
375 380 385	
aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac	1894
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp	
390 395 400	
tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc	1942
Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser	
405 410 415	
agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct	1990
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala	
420 425 430 435	
ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa	2038
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys	
440 445 450	
tgagtgcgac ggccggcaag ccccgctccc gaatt	2073

<210> 143

<211> 470

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed heavy chain of humanized anti-Fas antibody

<400> 143

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
-15 -10 -5

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
-1 1 5 10

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
15 20 25

Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Ala Pro Gly Gln Gly Leu  
30 35 40 45

Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn  
50 55 60

Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Thr Ser  
65 70 75

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val  
80 85 90

Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp  
95 100 105

Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
110 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
145 150 155

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
160 165 170

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
175 180 185

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
190 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro  
210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
225 230 235

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
240 245 250

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
255 260 265

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
270 275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
305 310 315

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
320 325 330

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
335 340 345

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
350 355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
400 405 410

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
415 420 425

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
430 435 440 445

Ser Leu Ser Pro Gly Lys  
450

<210> 144

<211> 2073

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed DNA  
encoding the heavy chain of a humanized anti-Fas  
antibody

<220>

<221> sig peptide

<222> (23)..(79)

<220>

<221> intron

<222> (737)..(1127)

<220>

<221> intron

<222> (1173) .. (1290)

<220>

<221> intron

<222> (1621) .. (1717)

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<222> (23) .. (736)

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<221> mat peptide

<222> (80) .. (736)

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<221> mat peptide

<222> (1291) .. (1620)

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<221> mat peptide

<222> (1718) .. (2038)

<220>

<221> CDS

<222> (23) .. (736)

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<221> CDS

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<221> CDS

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<220>

<221> CDS

<222> (1718) .. (2038)



Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr
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Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val
200					205					210				215	
gac	aag	aga	gtt	ggtgagaggc	cagcacaggg	agggagggtg	tctgctggaa								724
Asp	Lys	Arg	Val												776
gccaggctca	gcgctcctgc	ctggacgcatt	cccggtatg	cagtcccagt	ccagggcagc	836									
aaggcaggcc	ccgtctgcct	cttcacccgg	aggcctctgc	ccgccccact	catgctcagg	896									
gagagggtct	tctggctttt	tccccaggct	ctgggcaggc	acaggctagg	tgccccta	956									
ccaggccctg	cacacaaagg	ggcaggtgct	gggctcagac	ctgccaagag	ccatatccgg	1016									
gaggaccctg	cccctgaccc	aagcccaccc	caaaggccaa	actctccact	ccctcagctc	1076									
ggacaccttc	tctcctccca	gattccagta	actcccaatc	ttctctctgc	a gag ccc	1133									
					Glu Pro										
					220										
aaa	tct	tgt	gac	aaa	act	cac	aca	tgc	cca	ccg	tgc	cca	ggtaagccag		1182
Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Cys	Pro			
225					230										
cccaggcctc	gccctccagc	tcaaggcggg	acaggtgccc	tagatagcc	tgcattcagg	1242									
gacaggcccc	agccgggtgc	tgacacgtcc	acctccatct	cttcctca	gca cct gaa	1299									
					Ala Pro Glu										
					235										
ctc	ctg	ggg	gga	ccg	tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	ccc	aag	gac
Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp
240					245							250			1347
acc	ctc	atg	atc	tcc	cg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	gac
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp
255					260						265				1395
gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	ggc
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly
270					275					280				285	1443
gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	ccg	cg	gag	gag	cag	tac	aac
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn
290					295						300				1491
agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	cag	gac	tgg
Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp
305					310						315				1539

ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca	1587		
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro			
320	325	330	
gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggtgggaccc gtggggtgcg	1640		
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys			
335	340		
aggcccacat ggacagaggc cggctcgcc caccctctgc cctgagagtg accgctgtac	1700		
caacctctgt ccctaca ggg cag ccc cga gaa cca cag gtg tac acc ctg	1750		
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu			
345	350	355	
ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg acc tgc	1798		
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys			
360	365	370	
ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc	1846		
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser			
375	380	385	
aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac	1894		
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp			
390	395	400	
tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc	1942		
Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser			
405	410	415	
agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct	1990		
Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala			
420	425	430	435
ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa	2038		
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys			
440	445	450	
tgagtgcgac ggccggcaag ccccgctccc gaatt	2073		

<210> 145

<211> 470

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed heavy chain of humanized anti-Fas antibody

<400> 145

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
-15 -10 -5

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
-1 1 5 10

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
15 20 25

Thr Ser Tyr Trp Met Gln Trp Val Lys Gln Ala Pro Gly Gln Gly Leu  
30 35 40 45

Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn  
50 55 60

Gln Lys Phe Lys Gly Lys Ala Thr Ile Thr Val Asp Thr Ser Thr Ser  
65 70 75

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val  
80 85 90

Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp  
95 100 105

Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
110 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
145 150 155

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
160 165 170

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
175 180 185

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
190 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro  
210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
225 230 235

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
240 245 250

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
255 260 265

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
270 275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
305 310 315

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
320 325 330

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
335 340 345

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
350 355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
400 405 410

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
415 420 425

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
430 435 440 445

Ser Leu Ser Pro Gly Lys  
450

<210> 146

<211> 2073

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed DNA  
encoding the heavy chain of a humanized anti-Fas  
antibody

<220>

<221> sig peptide

<222> (23)..(79)

<220>

<221> intron

<222> (737)..(1127)

<220>

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<222> (1173)..(1290)

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<221> intron

<222> (1621)..(1717)

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<221> exon

<222> (23)..(736)

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<221> exon

<222> (1128)..(1172)

<220>

<221> exon

<222> (1291)..(1620)

<220>

<221> exon

<222> (1718)..(2038)

<220>

<221> mat peptide

<222> (80)..(736)

<220>

<221> mat peptide

<222> (1128)..(1172)

<220>

<221> mat peptide

<222> (1291)..(1620)

<220>

<221> mat peptide

<222> (1718)..(2038)

<220>

<221> CDS

<222> (23)..(736)

<220>

<221> CDS

<222> (1128)..(1172)

<220>

<221> CDS

<222> (1291)..(1620)

<220>

<221> CDS

<222> (1718)..(2038)



Tyr	Ser	Leu	Ser	Ser	Val	Val	Thr	Val	Pro	Ser	Ser	Ser	Leu	Gly	Thr	
185					190					195						
cag	acc	tac	atc	tgc	aac	gtg	aat	cac	aag	ccc	agc	aac	acc	aag	gtg	724
Gln	Thr	Tyr	Ile	Cys	Asn	Val	Asn	His	Lys	Pro	Ser	Asn	Thr	Lys	Val	
200					205					210					215	
gac	aag	aga	gtt	ggtgagagggc	cagcacaggg	agggagggtg	tctgctggaa									776
Asp	Lys	Arg	Val													
gccaggctca	gcgctcctgc	ctggacgcat	cccggtatg	cagtcccagt	ccagggcagc	836										
aaggcaggcc	ccgtctgcct	cttcacccgg	aggcctctgc	ccgccccact	catgctcagg	896										
gagagggtct	tctggcttt	tccccaggct	ctgggcaggc	acaggctagg	tgccccta	956										
ccaggccctg	cacacaaagg	ggcaggtgct	gggctcagac	ctgccaagag	ccatatccgg	1016										
gaggaccctg	cccctgaccc	aagcccaccc	caaaggccaa	actctccact	ccctcagctc	1076										
ggacacccctc	tctcctccca	gattccagta	actcccaatc	ttctctctgc	a	gag	ccc									1133
						Glu	Pro									
						220										
aaa	tct	tgt	gac	aaa	act	cac	aca	tgc	cca	ccg	tgc	cca	ggtaagccag			1182
Lys	Ser	Cys	Asp	Lys	Thr	His	Thr	Cys	Pro	Pro	Pro	Cys	Pro			
225					230											
cccaggcctc	gccctccagc	tcaaggcggg	acaggtgccc	tagatagcc	tgc	atccagg										1242
gacaggcccc	agccgggtgc	tgacacgtcc	acctccatct	cttcctca	gca	cct	gaa									1299
						Ala	Pro	Glu								
						235										
ctc	ctg	ggg	gga	ccg	tca	gtc	ttc	ctc	ttc	ccc	cca	aaa	ccc	aag	gac	1347
Leu	Leu	Gly	Gly	Pro	Ser	Val	Phe	Leu	Phe	Pro	Pro	Lys	Pro	Lys	Asp	
240					245					250						
acc	ctc	atg	atc	tcc	cg	acc	cct	gag	gtc	aca	tgc	gtg	gtg	gtg	gac	1395
Thr	Leu	Met	Ile	Ser	Arg	Thr	Pro	Glu	Val	Thr	Cys	Val	Val	Val	Asp	
255					260					265						
gtg	agc	cac	gaa	gac	cct	gag	gtc	aag	ttc	aac	tgg	tac	gtg	gac	ggc	1443
Val	Ser	His	Glu	Asp	Pro	Glu	Val	Lys	Phe	Asn	Trp	Tyr	Val	Asp	Gly	
270					275					280					285	
gtg	gag	gtg	cat	aat	gcc	aag	aca	aag	ccg	cg	gag	gag	cag	tac	aac	1491
Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	
290					295					295					300	
agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	cag	gac	tgg	1539
Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	
305					310					310					315	

ctg aat ggc aag gag tac aag tgc aag gtc tcc aac aaa gcc ctc cca	1587																																																																				
Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro																																																																					
320	325	330		gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggtgggaccc gtggggtgcg	1640	Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys		335	340	aggcccacat ggacagaggc cggctcggcc caccctctgc cctgagagtg accgctgtac	1700	caacctctgt ccctaca ggg cag ccc cga gaa cca cag gtg tac acc ctg	1750	Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu		345	350	355		ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg acc tgc	1798	Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys		360	365	370		ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc	1846	Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser		375	380	385		aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac	1894	Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp		390	395	400		tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc	1942	Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser		405	410	415		agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct	1990	Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala		420	425	430	435	ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa	2038	Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		440	445	450		tgagtgcgac ggccggcaag ccccgctccc gaatt	2073
330																																																																					
gcc ccc atc gag aaa acc atc tcc aaa gcc aaa ggtgggaccc gtggggtgcg	1640																																																																				
Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys																																																																					
335	340																																																																				
aggcccacat ggacagaggc cggctcggcc caccctctgc cctgagagtg accgctgtac	1700																																																																				
caacctctgt ccctaca ggg cag ccc cga gaa cca cag gtg tac acc ctg	1750																																																																				
Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr Leu																																																																					
345	350	355		ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg acc tgc	1798	Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys		360	365	370		ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc	1846	Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser		375	380	385		aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac	1894	Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp		390	395	400		tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc	1942	Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser		405	410	415		agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct	1990	Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala		420	425	430	435	ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa	2038	Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		440	445	450		tgagtgcgac ggccggcaag ccccgctccc gaatt	2073																
355																																																																					
ccc cca tcc cgg gag gag atg acc aag aac cag gtc agc ctg acc tgc	1798																																																																				
Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser Leu Thr Cys																																																																					
360	365	370		ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc	1846	Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser		375	380	385		aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac	1894	Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp		390	395	400		tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc	1942	Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser		405	410	415		agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct	1990	Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala		420	425	430	435	ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa	2038	Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		440	445	450		tgagtgcgac ggccggcaag ccccgctccc gaatt	2073																								
370																																																																					
ctg gtc aaa ggc ttc tat ccc agc gac atc gcc gtg gag tgg gag agc	1846																																																																				
Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu Ser																																																																					
375	380	385		aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac	1894	Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp		390	395	400		tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc	1942	Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser		405	410	415		agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct	1990	Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala		420	425	430	435	ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa	2038	Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		440	445	450		tgagtgcgac ggccggcaag ccccgctccc gaatt	2073																																
385																																																																					
aat ggg cag ccg gag aac aac tac aag acc acg cct ccc gtg ctg gac	1894																																																																				
Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu Asp																																																																					
390	395	400		tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc	1942	Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser		405	410	415		agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct	1990	Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala		420	425	430	435	ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa	2038	Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		440	445	450		tgagtgcgac ggccggcaag ccccgctccc gaatt	2073																																								
400																																																																					
tcc gac ggc tcc ttc ctc tat agc aag ctc acc gtg gac aag agc	1942																																																																				
Ser Asp Gly Ser Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys Ser																																																																					
405	410	415		agg tgg cag cag ggg aac gtc ttc tca tgc tcc gtg atg cat gag gct	1990	Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu Ala		420	425	430	435	ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa	2038	Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		440	445	450		tgagtgcgac ggccggcaag ccccgctccc gaatt	2073																																																
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420	425	430	435	ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa	2038	Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys		440	445	450		tgagtgcgac ggccggcaag ccccgctccc gaatt	2073																																																								
430	435																																																																				
ctg cac aac cac tac acg cag aag agc ctc tcc ctg tcc ccg ggt aaa	2038																																																																				
Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly Lys																																																																					
440	445	450		tgagtgcgac ggccggcaag ccccgctccc gaatt	2073																																																																
450																																																																					
tgagtgcgac ggccggcaag ccccgctccc gaatt	2073																																																																				

<210> 147

<211> 470

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed heavy chain of humanized anti-Fas antibody

<400> 147

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
-15 -10 -5

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
-1 1 5 10

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
15 20 25

Thr Ser Tyr Trp Met Gln Trp Val Arg Gln Ala Pro Gly Gln Gly Leu  
30 35 40 45

Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn  
50 55 60

Gln Lys Phe Lys Gly Lys Ala Thr Leu Thr Val Asp Thr Ser Thr Ser  
65 70 75

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val  
80 85 90

Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp  
95 100 105

Val Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
110 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
145 150 155

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
160 165 170

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
175 180 185

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
190 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro  
210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
225 230 235

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
240 245 250

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
255 260 265

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
270 275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
305 310 315

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
320 325 330

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
335 340 345

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
350 355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
400 405 410

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
415 420 425

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
430 435 440 445

Ser Leu Ser Pro Gly Lys  
450

<210> 148  
<211> 38  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the heavy chain  
of a humanized anti-Fas antibody

<400> 148  
ccaaagcttgg cttgacctca ccatggatg gagctgta

38

<210> 149  
<211> 40  
<212> DNA  
<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 149

agtgggtaaa acaggcccct ggacagggac ttgagtgat

40

<210> 150

<211> 40

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 150

atccactcaa gtcctgtcc agggcctgt tttaccact

40

<210> 151

<211> 64

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 151

aagaccgatg ggccttggt ggaggctgag gagacggta ccagtgtacc ttggccccag 60

acat

64

<210> 152

<211> 39

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of a humanized anti-Fas antibody

<400> 152

gttcaagggc aaggccacaa taactgtaga cacatccgc

39

<210> 153

<211> 39

<212> DNA  
<213> Artificial Sequence  
  
<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the heavy chain  
of a humanized anti-Fas antibody

<400> 153  
gcggatgtgt ctacagttat tgtggccttg cccttgaac

39

<210> 154  
<211> 40  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the heavy chain  
of a humanized anti-Fas antibody

<400> 154  
agtgggtacg acaggcccct ggacaaggac ttgagtggt

40

<210> 155  
<211> 40  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the heavy chain  
of a humanized anti-Fas antibody

<400> 155  
atccactcaa gtccttgtcc aggggcctgt cgtacccact

40

<210> 156  
<211> 2077  
<212> DNA  
<213> Artificial Sequence

<220>  
<221> sig peptide  
<222> (27)..(83)

<220>  
<221> intron  
<222> (741)..(1131)

<220>

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> <221> intron  
<222> (1177) .. (1294)

<220>  
<221> intron  
<222> (1625) .. (1725)

<220>  
<221> exon  
<222> (27) .. (740)

<220>  
<221> exon  
<222> (1132) .. (1176)

<220>  
<221> exon  
<222> (1295) .. (1624)

<220>  
<221> exon  
<222> (1722) .. (2042)

<220>  
<221> mat peptide  
<222> (84) .. (740)

<220>  
<221> mat peptide  
<222> (1132) .. (1176)

<220>  
<221> mat peptide  
<222> (1295) .. (1624)

<220>  
<221> mat peptide  
<222> (1722) .. (2042)

<220>  
<221> CDS  
<222> (27) .. (740)

<220>  
<221> CDS  
<222> (1132) .. (1176)

<220>  
<221> CDS  
<222> (1295) .. (1624)

<220>  
<221> CDS  
<222> (1722) .. (2042)

<220>

<223> Description of Artificial Sequence: Designed DNA encoding the heavy chain of humanized anti-Fas antibody

<400> 156

gggcgaaagc ttggcttgac ctcacc atg gga tgg agc tgt atc atc ctc ttc 53  
Met Gly Trp Ser Cys Ile Ile Leu Phe  
-15

tct ggg gct gag gtc aag aag cct ggg gct tca gtg aag gtg tcc tgc 149  
 Ser Gly Ala Glu Val Lys Lys Pro Gly Ala Ser Val Lys Val Ser Cys  
           10                 15                 20

aag	gct	tct	ggc	tac	acc	ttc	acc	agc	tac	tgg	atg	cag	tgg	gta	cga	197
Lys	Ala	Ser	Gly	Tyr	Thr	Phe	Thr	Ser	Tyr	Trp	Met	Gln	Trp	Val	Arg	
25					30					35						

cag	gcc	cct	gga	cag	ggc	ctt	gag	tgg	atg	gga	gag	att	gat	cct	tct	245
Gln	Ala	Pro	Gly	Gln	Gly	Leu	Glu	Trp	Met	Gly	Glu	Ile	Asp	Pro	Ser	
40				45						50						

act cga gac aca tcc act agc aca gcc tac atg gag ctc agc agc ctg 341  
 Thr Arg Asp Thr Ser Thr Ser Thr Ala Tyr Met Glu Leu Ser Ser Leu  
 75 80 85

```

aga tct gag gac acg gcg gtc tat tac tgt gca aga aat agg gac tat 389
Arg Ser Glu Asp Thr Ala Val Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr
         90          95          100

```

```

agt aac aac tgg tac ttc gat gtc tgg ggc gaa ggg acc ctg gtc acc 437
Ser Asn Asn Trp Tyr Phe Asp Val Trp Gly Glu Gly Thr Leu Val Thr
105          110          115

```

gtc tcc tca gcc tcc acc aag ggc cca tcg gtc ttc ccc ctg gca ccc 485  
 Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe Pro Leu Ala Pro  
 120 125 130

tcc	tcc	aag	agc	acc	tct	ggg	ggc	aca	gcg	gcc	ctg	ggc	tgc	ctg	gtc	533
Ser	Ser	Lys	Ser	Thr	Ser	Gly	Gly	Thr	Ala	Ala	Leu	Gly	Cys	Leu	Val	
135					140					145					150	

aag	gac	tac	ttc	ccc	gaa	ccg	gtg	acg	gtg	tcg	tgg	aac	tca	ggc	gcc	581
Lys	Asp	Tyr	Phe	Pro	Glu	Pro	Val	Thr	Val	Ser	Trp	Asn	Ser	Gly	Ala	
				155				160						165		

ctg acc agc ggc gtg cac acc ttc ccg gct gtc cta cag tcc tca gga	629
Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu Gln Ser Ser Gly	
170	175
180	
ctc tac tcc ctc agc agc gtg gtg acc gtg ccc tcc agc agc ttg ggc	677
Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser Ser Ser Leu Gly	
185	190
195	
acc cag acc tac atc tgc aac gtg aat cac aag ccc agc aac acc aag	725
Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro Ser Asn Thr Lys	
200	205
210	
gtg gac aag aga gtt ggtgagaggc cagcacaggg agggagggtg tctgctggaa	780
Val Asp Lys Arg Val	
215	
gccaggctca gcgctcctgc ctggacgcat cccggctatg cagtcccagt ccagggcagc	840
aaggcaggcc ccgtctgcct cttcacccgg aggccctctgc ccgccccact catgctcagg	900
gagagggtct tctggctttt tccccaggct ctgggcaggc acaggctagg tgcccctaac	960
ccaggccctg cacacaaagg ggcaggtgct gggctcagac ctgccaagag ccatatccgg	1020
gaggaccctg cccctgaccc aagcccaccc caaaggccaa actctccact ccctcagctc	1080
ggacacccccc tctcctccca gattccagta actcccaatc ttctctctgc a gag ccc	1137
Glu Pro	
220	
aaa tct tgt gac aaa act cac aca tgc cca ccg tgc cca ggtaagccag	1186
Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro	
225	230
cccaggcctc gccctccagc tcaaggcggg acaggtgccc tagatagcc tgcataccagg	1246
gacaggcccc agccgggtgc tgacacgtcc acctccatct cttcctca gca cct gaa	1303
Ala Pro Glu	
235	
ctc ctg ggg gga ccg tca gtc ttc ctc ttc ccc cca aaa ccc aag gac	1351
Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp	
240	245
250	
acc ctc atg atc tcc cgg acc cct gag gtc aca tgc gtg gtg gtg gac	1399
Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp	
255	260
265	
gtg agc cac gaa gac cct gag gtc aag ttc aac tgg tac gtg gac ggc	1447
Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly	
270	275
280	285
gtg gag gtg cat aat gcc aag aca aag ccg cgg gag gag cag tac aac	1495

Val	Glu	Val	His	Asn	Ala	Lys	Thr	Lys	Pro	Arg	Glu	Glu	Gln	Tyr	Asn	
290									295						300	
agc	acg	tac	cgt	gtg	gtc	agc	gtc	ctc	acc	gtc	ctg	cac	cag	gac	tgg	1543
Ser	Thr	Tyr	Arg	Val	Val	Ser	Val	Leu	Thr	Val	Leu	His	Gln	Asp	Trp	
305								310						315		
ctg	aat	ggc	aag	gag	tac	aag	tgc	aag	gtc	tcc	aac	aaa	gcc	ctc	cca	1591
Leu	Asn	Gly	Lys	Glu	Tyr	Lys	Cys	Lys	Val	Ser	Asn	Lys	Ala	Leu	Pro	
320								325						330		
gcc	ccc	atc	gag	aaa	acc	atc	tcc	aaa	gcc	aaa	ggtgtggaccc	gtggggtgcg	1644			
Ala	Pro	Ile	Glu	Lys	Thr	Ile	Ser	Lys	Ala	Lys						
335								340								
aggcccacat	ggacagaggc	cggtcgcc	caccctctgc	cctgagagtgc	accgctgtac											1704
caacctctgt	ccctaca	ggg	cag	ccc	cga	gaa	cca	cag	gtg	tac	acc	ctg				1754
		Gly	Gln	Pro	Arg	Glu	Pro	Gln	Val	Tyr	Thr	Leu				
345								350						355		
ccc	cca	tcc	cgg	gag	gag	atg	acc	aag	aac	cag	gtc	agc	ctg	acc	tgc	1802
Pro	Pro	Ser	Arg	Glu	Glu	Met	Thr	Lys	Asn	Gln	Val	Ser	Leu	Thr	Cys	
360								365						370		
ctg	gtc	aaa	ggc	ttc	tat	ccc	agc	gac	atc	gcc	gtg	gag	tgg	gag	agc	1850
Leu	Val	Lys	Gly	Phe	Tyr	Pro	Ser	Asp	Ile	Ala	Val	Glu	Trp	Glu	Ser	
375								380						385		
aat	ggg	cag	ccg	gag	aac	aac	tac	aag	acc	acg	cct	ccc	gtg	ctg	gac	1898
Asn	Gly	Gln	Pro	Glu	Asn	Asn	Tyr	Lys	Thr	Thr	Pro	Pro	Val	Leu	Asp	
390								395						400		
tcc	gac	ggc	tcc	ttc	ttc	ctc	tat	agc	aag	ctc	acc	gtg	gac	aag	agc	1946
Ser	Asp	Gly	Ser	Phe	Phe	Leu	Tyr	Ser	Lys	Leu	Thr	Val	Asp	Lys	Ser	
405								410						415		
agg	tgg	cag	cag	ggg	aac	gtc	ttc	tca	tgc	tcc	gtg	atg	cat	gag	gct	1994
Arg	Trp	Gln	Gln	Gly	Asn	Val	Phe	Ser	Cys	Ser	Val	Met	His	Glu	Ala	
420								425						430		435
ctg	cac	aac	cac	tac	acg	cag	aag	agc	ctc	tcc	ctg	tcc	ccg	ggt	aaa	2042
Leu	His	Asn	His	Tyr	Thr	Gln	Lys	Ser	Leu	Ser	Leu	Ser	Pro	Gly	Lys	
440								445						450		
tgagtgcgac	ggccggcaag	ccccgctccc	gaatt													2077

<210> 157

<211> 470

<212> PRT

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: Designed  
heavy chain of humanized anti-Fas antibody

<400> 157

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly  
-15 -10 -5

Val His Ser Gln Val Gln Leu Val Gln Ser Gly Ala Glu Val Lys Lys  
-1 1 5 10

Pro Gly Ala Ser Val Lys Val Ser Cys Lys Ala Ser Gly Tyr Thr Phe  
15 20 25

Thr Ser Tyr Trp Met Gln Trp Val Arg Gln Ala Pro Gly Gln Gly Leu  
30 35 40 45

Glu Trp Met Gly Glu Ile Asp Pro Ser Asp Ser Tyr Thr Asn Tyr Asn  
50 55 60

Gln Lys Phe Lys Gly Arg Val Thr Ile Thr Arg Asp Thr Ser Thr Ser  
65 70 75

Thr Ala Tyr Met Glu Leu Ser Ser Leu Arg Ser Glu Asp Thr Ala Val  
80 85 90

Tyr Tyr Cys Ala Arg Asn Arg Asp Tyr Ser Asn Asn Trp Tyr Phe Asp  
95 100 105

Val Trp Gly Glu Gly Thr Leu Val Thr Val Ser Ser Ala Ser Thr Lys  
110 115 120 125

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly  
130 135 140

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro  
145 150 155

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr  
160 165 170

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val  
175 180 185

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn  
190 195 200 205

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro  
210 215 220

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu  
225 230 235

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp  
240 245 250

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp  
255 260 265

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly  
270 275 280 285

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn  
290 295 300

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp  
305 310 315

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro  
320 325 330

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu  
335 340 345

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn  
350 355 360 365

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile  
370 375 380

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr  
385 390 395

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys  
400 405 410

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys  
415 420 425

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu  
430 435 440 445

Ser Leu Ser Pro Gly Lys  
450

<210> 158

<211> 29

<212> DNA

<213> Artificial Sequence

<220>

<223> Description of Artificial Sequence: PCR primer to  
amplify a fragment of DNA encoding the heavy chain  
of humanized anti-Fas antibody

<400> 158

gatgcagtgg gtacgacagg cccctggac

<210> 159  
<211> 29  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of humanized anti-Fas antibody

<400> 159  
gtccaggggc ctgtcgtaacc cactgcac 29

<210> 160  
<211> 33  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of humanized anti-Fas antibody

<400> 160  
caagggccgg gtcacaatca ctcgagacac atc 33

<210> 161  
<211> 33  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer to amplify a fragment of DNA encoding the heavy chain of humanized anti-Fas antibody

<400> 161  
gatgtgtctc gagtgattgt gacccggccc ttg 33

<210> 162  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of humanized anti-Fas antibody

<400> 162

ctacaatcaa aagttcaagg

20

<210> 163  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of humanized anti-Fas antibody

<400> 163  
gactatagta acaactggta c

21

<210> 164  
<211> 21  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of humanized anti-Fas antibody

<400> 164  
gtaccagttt ttactatagt c

21

<210> 165  
<211> 20  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: Sequencing primer for a DNA encoding the heavy chain of humanized anti-Fas antibody

<400> 165  
gcagcccagg gccgctgtgc

20